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JOINTED RUSHES OF THE OXFORD DISTRICT

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(With 7 figures in the text)

It has been realized for some time that the jointed rushes of the Oxford district, and in particular those of the *sylvaticus-articulatus* group, present certain difficulties in identification. It was decided, therefore, that they would provide interesting material for a combined cytological and taxonomic investigation. The work was begun in the late autumn of 1938, when the fruiting shoots of that season were already dead and in bad condition for critical examination, but since one of us (E. W. T.) had only one more academic year in Oxford, it was important to begin the cytological studies at once.

Cytological technique

Actively growing root tips were used for somatic chromosome counts. These could conveniently be obtained by placing the plant, from which the soil had been washed, in a jar of water, changed daily. Under favourable conditions, an abundant supply of new root tips was available in 3–4 days; during the winter 7–10 days was necessary. The root tips were fixed in La Cour 2 BE fixative.

Buds for the study of meiotic divisions were first dipped in Carnoy's fluid for several seconds and then fixed in either La Cour 2 BE, Navashin Graf or a fixative of the Navashin type in use at Oxford, with 1·5 c.c. 10 % aqueous chromic acid, 10 % acetic acid, 8·3 c.c. 25 % formalin and 16·2 c.c. distilled water.

The material was embedded in paraffin (M.P. 49° C.) and sectioned with a Spencer rotary microtome at a thickness of 10 μ for root tips and 15 μ for buds. The chromosomes were stained by Newton's gentian violet method. The best results were obtained by staining in an 0·5 % aqueous solution of gentian violet for about 30 min. and then destaining for only a few seconds in the alcohols and clove oil.

The preparations were studied with a Zeiss binocular microscope, using a Zeiss 2 mm. objective or a Zeiss apochromatic 1·5 mm. objective, together with compensating 15 × eyepieces.

Cytological results

Since this problem was begun late in the autumn of 1938 and good inflorescences and capsules were not obtainable, identification was difficult, and it was therefore deemed advisable to collect samples of the various populations for somatic chromosome counts. Chromosome counts of $2n = 40$, 60, and 80 were obtained. The

chromosomes are very small, ranging in size from 0·25 to 1·25 μ in length in each of the three groups; no appreciable differences could be detected (Fig. 1). From the limited morphological data then available it was provisionally concluded that for *Juncus articulatus* as described in British floras, $2n=80$; for *J. sylvaticus*, $2n=40$; and that the form with $2n=60$ must be interpreted as a hybrid between these two species.

Before the flowering season began the range of plants studied was extended and intensified, and it became possible with few exceptions to classify the specimens by their vegetative morphology before the counts were made. Toward the end of June a population near Wytham which had been tentatively considered as composed of

Fig. 1. Mitotic metaphase plates from root tips. ×4100. *a, Juncus sylvaticus*; $n=40$. *b, J. sylvaticus × J. articulatus*; $n=60$ *c, J. articulatus*; $n=80$.

Fig. 2. Trivalents from metaphase I in pollen mother cells of *J. sylvaticus × J. articulatus*.

hybrids aroused interest because of its unusual sturdiness and later flowering period. It was found that for this population $2n=80$, but it was undoubtedly distinct from *J. articulatus* as judged by its morphology. It was designated as "large 80". The morphology of all four forms and the status of "large 80" are discussed in later sections. Brief details of the plants studied are given in the Appendix (p. 14).

Studies of meiosis in the form with 60 chromosomes confirmed the view that it is a hybrid. There seems to be a periodicity in the flowering among these rushes, so that in the time available preparations of all stages of meiosis were not obtained. Pairing with chiasma formation takes place presumably between 20 *J. sylvaticus* chromosomes and chromosomes contributed by *J. articulatus*, yielding from 16 to 20 bivalents and 4 to 0 trivalents (Fig. 2). The remaining 16 to 20 univalents are slow in coming to the plate during metaphase and lag at anaphase, undergoing

division on the plate after the bivalents and trivalents have separated and have passed to the poles. The complete sterility of this form is in accordance with these observations and the postulate of hybrid origin. Mere observations on pollen tetrads in aceto-carmine are misleading; about 80 % appear normal.

The fact that this homology obtains between the *J. sylvaticus* and *J. articulatus* complement in the hybrid, as indicated by the number of bivalents and trivalents, is evidence in favour of considering *J. articulatus* as having originated by tetraploidy from *J. sylvaticus* or from a hybrid having *J. sylvaticus* as one parent. Even if the assumption is made that the occurrence of bivalents is explained by the autosyndesis of *J. articulatus* chromosomes, allosyndesis must still be involved in the formation of trivalents. It is hoped that future studies on multivalent formation in *J. articulatus* will clarify this point. From a large number of second anaphases examined and from the high fertility of *J. articulatus* it is concluded that meiosis is well regulated. In several cells a few chromosomes were seen to lag, but they were not being excluded from the nuclei in the tetrads.

Ecology and morphology of the four types

(1) Juncus articulatus *L.* (Juncus lampocarpus *Ehrh.*); $2n=80$

In the Oxford district *J. articulatus* is a common and rather variable plant. It is found in the wettest alluvial meadows but is abundant only in those which are grazed, not in mowing meadows. It is a frequent plant of the seepage lines at the junction of the permeable Calcareous Grit and the Oxford Clay, and of drains cut in clay. It is especially characteristic, in prostrate forms, of paths and trodden places which have standing water except during summer months, and its occurrences on calcareous peat are confined to paths. It appears to need a firm or finely divided substratum, and cannot survive in tall vegetation.

J. articulatus is distinguishable from *J. sylvaticus* and from their hybrids by the slender, hardly creeping rhizomes which give the plant a subcaespitose rather than creeping habit. Its aerial shoots are also more slender than in *J. sylvaticus*, and they are never rigidly erect in the Oxford district. Its leaves are usually more numerous (3–7) than those of *J. sylvaticus*, and are shorter, broader, less rigid, more flattened, more deeply ribbed and less shining. They are often curved inwards towards the stem. The plants flower in late June or early July, from 2 to 4 weeks earlier than *J. sylvaticus*. The inflorescence has long branches ascending at *c.* 30° with the vertical, the peduncles of the heads are long, and there are eight or fewer flowers per head. The flowers have inner and outer perianth segments about equally long, the inner with a broad and the outer with a narrow colourless transparent margin. The outer perianth segments are acute; the inner are usually acute, but may be rounded. The anthers are short, with their filaments as long as or longer than themselves. The stigma is white and the style is shorter than the ovary. The capsules are black and shining when ripe, and are very variable in shape, but are almost always shouldered, though acuminate capsules are occasionally found.

(2) Juncus sylvaticus *auct. mult. an etiam Reich.* (J. acutiflorus *Ehrh.*); $2n=40$

In its wider distribution *J. sylvaticus* is predominantly a plant of acid and rather base-poor habitats, showing a wide range of tolerance both of water-content of the soil and of physical nature of the substratum, but growing luxuriantly in wet acid peats which are not extremely oligotrophic. Here it is the moderately oligotrophic counterpart of the strongly eutrophic *J. obtusiflorus*. In the Oxford district, where oligotrophic conditions are very local, *J. sylvaticus* is not very abundant, and is a plant of drier habitats than *J. articulatus*. It is especially characteristic of certain of the drier alluvial mowing meadows (Pixey and Yarnton Meads), flooded for short periods during the winter, but becoming quite dry in a normal summer. It is not found in the drier meadows which are heavily grazed. In the strikingly zoned Junceta of the seepage lines where the Calcareous Grit overlies Oxford Clay, it occupies belts between *J. inflexus* and *J. obtusiflorus*, the former in drier and the latter in wetter peaty habitats.

J. sylvaticus has a strong, far-creeping rhizome, and its aerial shoots are tall and rigidly erect with only 2–4 long subterete leaves which are slender, rigid and shining. Their articulations are distinctly visible as dark lines even in the fresh leaves. The flowering period is later than in *J. articulatus*, and it is difficult to find ripe fruit before the end of August. The inflorescence branches are of two kinds. One or two of the strong basal branches ascend almost vertically, while numerous shorter branches spread at a wide angle. There are more flowers per head than in *J. articulatus*, but the individual flowers are smaller. Both outer and inner perianth segments are narrow and very acute, and the inner are conspicuously longer than the outer in most specimens, this difference being enhanced by the curling inwards or outwards of the tips of the outer segments. There is a quite narrow brownish transparent margin to the inner perianth segments. The stigmas are pink, the style is about as long as the ovary, and the stamens have long anthers with shorter filaments. The capsule is reddish or yellowish brown and is long-acuminate, usually with no appreciable shoulder.

(3) Juncus articulatus × J. sylvaticus; $2n=60$

The hybrid *J. articulatus* × *J. sylvaticus* has been found to be a very common plant in the neighbourhood of Oxford. Just above Godstow it occupies a narrow zone between populations of *J. articulatus* on the river bank and of *J. sylvaticus* in a fairly dry meadow which is not heavily grazed. This suggestion of demands intermediate between those of the parent types is confirmed by its abundance in mowing meadows (Wytham Meadows, Iffley Meadows) which are lower lying and more frequently flooded than those which carry *J. sylvaticus*, but from which *J. articulatus* appears to be excluded by taller-growing species. At Weston it occurs with *J. obtusiflorus* and *J. sylvaticus* in tall vegetation, the former in wetter and the latter in drier habitats than the hybrid. Here *J. articulatus* is confined to the margins of streams and the sides of paths. It is always present, and may be abundant, in

the seepage zones where Calcareous Grit overlies Oxford Clay, as at Hinksey and Wytham. Prostrate forms of the hybrid are also found commonly on paths in the large mowing meadows (Pixey Mead and Yarnton Mead).

Morphologically, the hybrid shows a considerable range in stature and habit, but is on the whole intermediate between its parents, bearing in mind the variability of *J. articulatus*. It has a creeping rhizome more slender than that of *J. sylvaticus* but much stouter and farther creeping than that of *J. articulatus*. Its aerial shoots are stouter than those of *J. articulatus* and are usually ascending, but are not stiffly erect. In some forms, especially of those of paths in the mowing meads, they may be almost prostrate. Each aerial stem bears 3–5 leaves which are intermediate in length between those of the parents, slender, somewhat compressed and shining. Flowering begins later than in *J. articulatus* but earlier than in *J. sylvaticus*. The inflorescence resembles that of *J. articulatus* in form, with the branches longer and more ascending than in *J. sylvaticus*. There are few flowers per head (3–6), and the individual flowers are larger than in either parent. The perianth segments are intermediate between those of the parents in shape and in width of the colourless margins. The inner and outer segments are about equal in length. The stigma is white, the style is usually long, and the anthers are long with short filaments. The capsule contains no seeds, and is usually small and almost hidden by the perianth segments, so that by contrast with the parents the inflorescences have a strikingly meagre appearance in late summer. Occasionally the capsules enlarge, when they resemble those of *J. articulatus* in form and may become black, but no seeds have been found even in these.

It seems to be especially characteristic of the hybrid, though not confined to it, that in some individuals there is a continued multiplication of the number of flowers per head, so that long after the flowering period has begun quite young flowers can still be found. In these multiplied heads certain of the flowers commonly proliferate, giving vegetative plantlets as in other viviparous rushes. This must constitute an efficient mode both of reproduction and of dispersal.

(4) "*Large* 80", $2n=80$

The main population of "large 80" was found in the wettest parts of the seepage zone between the upper and lower meadows on the south side of Hagley Pool, Wytham. Other small patches were found in the lower meadow, and a few plants at Weston among *J. sylvaticus*. The plants are very vigorous, being taller and stouter than any other jointed rushes of the district, except for *J. sylvaticus* at its most luxuriant. The rhizome is stout and creeping and the aerial shoots are stiffly erect. The 2–4 leaves are fairly long and rigid, but are broader and flatter than those of *J. sylvaticus*. As in the latter, the articulations can be seen as distinct dark lines in the fresh and turgid leaves. Flowering is late, again as in *J. sylvaticus*. The large inflorescence has steeply ascending long branches and divergent short branches as in *J. sylvaticus*, but the short branches are fewer in number and there are far fewer heads of flowers. The heads are very large, larger even than in *J. articulatus*. There are often several abortive flowers in the head. The perianth segments are all acute,

and are usually mucronate or cuspidate. The inner segments are both longer and broader than the outer and have a conspicuously broad colourless transparent margin. The tips of the outer segments are somewhat curled inwards or outwards, as in *J. sylvaticus*. The ripe capsule is chestnut-coloured and glossy, and is rather narrowly acuminate, though usually with a slight shoulder. It is much larger than in *J. sylvaticus*, and is only occasionally equalled in *J. articulatus*. There are about 19 seeds per capsule, as against *c.* 41 in *J. articulatus* and *c.* 12 in *J. sylvaticus*.

(5) *Note on the number of articulations per leaf*

It seemed possible at one stage of this work that the number of articulations per leaf, or their average distance apart, might prove to be a character of diagnostic value. Careful examination soon showed, however, that there is only a small range of variation (18–25) in the total number of articulations per leaf, despite the very considerable range in length of leaf. The same number was found in all four types described, and the assumption seems justified that there is no genetic differentiation in this respect. A consequence of this approximate constancy of number is that the average distance apart of the articulations is proportional to the length of the leaf, irrespective of the taxonomic status of the plant. It should be stated that the articulations are by no means evenly spaced, being close together at apex and base of the leaf limb and widest apart in the middle region. The average distance apart of the five articulations most nearly central was, however, found to conform to the same relation, that of direct proportionality to the length of the leaf.

It is interesting to compare this state of affairs with that in *J. obtusiflorus*, a jointed rush which is taxonomically distant from *J. articulatus* and *J. sylvaticus*, and which does not form hybrids with them. Here the articulations are less distinct, many of the septa being incomplete. Their number is far greater, 35–50 per leaf, and the average distance apart of the five central articulations is much smaller for leaves of the same length. It may be inferred that in this taxonomically remote species leaf morphology is governed by a different genetic complex from that operative in the *articulatus-sylvaticus* group.

Summary of descriptions

Table 1 and Figs. 3–7 summarize and illustrate the foregoing descriptions. Where a range followed by a figure in brackets is given in Table 1, the former includes approximately 90 % of the observations, and the latter is a mean or modal value. Fig. 3 is based on the modal values for leaf number and the mean values for overall length of flowering shoot and lengths of the leaves. The mean lengths of the second youngest leaves are also given in the table, since they are less variable and more characteristic of the type of rush than are the lengths of any of the other leaves. It should be emphasized that quantitative observations were made on small ($n = 30$–60) samples collected in the Oxford district, and are introduced only to give greater definiteness to the descriptions.

Table 1. *Summary of characters of the four described types*

	1. *J. articulatus*	2. *J. sylvaticus*	3. *J. articulatus* × *J. sylvaticus*	4. "Large 80"
No. of chromosomes	80	40	60	80
Rhizome system	Shortly creeping or sub-caespitose	Far creeping	Far creeping	Far creeping
Diam. of rhizome	1·5–3 mm.	5–7 mm.	3–5 mm.	5–6 mm.
Aerial shoot	Decumbent	Stiffly erect	Slightly decumbent	Stiffly erect
Length of flowering shoot	36 cm.	72 cm.	50 cm.	65 cm.
No. of leaves	4–7 (5)	2–4 (3)	3–5 (4)	2–4 (3)
Length of blade of 2nd leaf from apex	9·3 cm.	28·0 cm.	13·8 cm.	21 6 cm.
Appearance of leaves	Deeply ribbed, dull, soft, often curved	Smooth, shining, stiff, straight	Smooth, shining, fairly stiff, occasionally curved	Smooth or slightly ribbed, rather dull, stiff, straight
Flattening of leaves	Strongly compressed	Subterete	Subterete	Moderately compressed
Visibility of articulations of fresh leaves	Inconspicuous	Conspicuous	Inconspicuous	Conspicuous
Flowering period	Early (from mid-June)	Late (from mid-July)	Intermediate (July)	Late (from mid-July)
Inflorescence branches	Few; all ascending at about 30° to vertical	Many; long basal branches ascending almost vertically; shorter branches widely spreading	Fairly numerous; 1–2 long and ascending almost vertically; shorter branches spreading at about 45°	Few; long branches ascending almost vertically; shorter branches spreading at 45° or more widely
Peduncles of heads	Long	Short	Long	Long
Heads of flowers	Few	Very many	Few to many	Few
No. of flowers per head	4–8 (5·5)	6–12 (8·6)	3–6 (4·4)	14–18 (15·9)
Size of flower	Large	Small	Large	Large
Length of inner P.	= Outer	> Outer	= Outer	> Outer
Transparent margin of inner P.	Colourless, broad	Brownish, narrow	Colourless, narrow	Colourless, broad
Tips of outer P.	Straight	Curved outwards	Slightly curved	Slightly curved
Length of anther in relation to filament	Short	Long	Fairly long	Fairly long
Length of style	Short	Long	Fairly long	Fairly long
Length of ripe capsule	3·6 mm.	3·0 mm	—	4 0 mm.
Colour of ripe capsule	Shining black	Brown	—	Shining chestnut brown
No. of seeds per capsule	41·4	12·4	0	19·0

NOTE. All data are based on samples collected in the Oxford district, and should not be regarded as applicable to other districts. In particular, *J. articulatus* may have much smaller average dimensions and an erect, not decumbent, habit.

RECORDS OF THE HYBRID OUTSIDE THE OXFORD DISTRICT

Chromosome counts have shown the existence of the hybrid in Matley Bog, Hampshire, on the east side of the New Forest. During a visit paid to this bog in July 1938 it was found difficult to identify some of the jointed rushes growing there. Mr T. Moir kindly brought a small collection of living plants from the bog in April 1939, and one of these had 60 chromosomes. The locality was revisited in July 1939, and plants identified morphologically as hybrids were found growing with the

parent species on the roadside. The jointed rushes in the bog were chiefly *J. sylvaticus*, but a few undoubted hybrids were seen, and also a patch of plants which, although they had slender shining leaves, were believed to be *J. articulatus* because they had well-formed black capsules. On closer examination of specimens taken back to Oxford it was found that the styles and anthers were unusually long for *J. articulatus*, and it was felt desirable to make a chromosome count. The number found was 60, and it was only then discovered that the capsules, though large and black, had no seeds.

A specimen sent by Dr E. F. Warburg from the pine wood on the south side of Loch Morlich in Speyside, Inverness, was also identified morphologically as a hybrid, the identification being later confirmed cytologically.

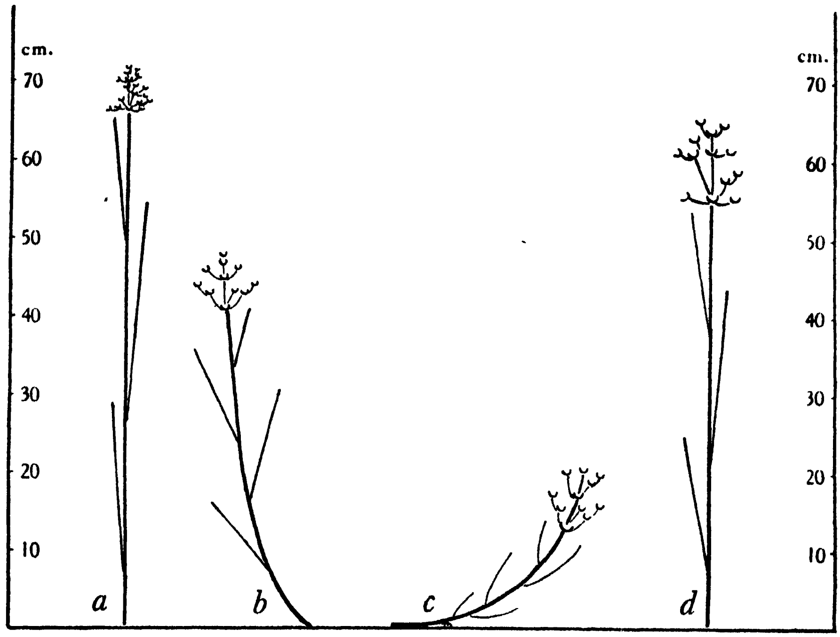

Fig. 3. Scale diagrams of the four described types, with dimensions based on means of samples collected near Oxford. *a*, *J. sylvaticus*. *b*, *J. sylvaticus* × *J. articulatus*. *c*, *J. articulatus*, *d*, "Large 80".

No other counts have been made, but hybrids have been recognized near St Kew Highway in Cornwall, near Clovelly and on Dartmoor in Devon, on Exmoor in west Somerset, and on Studland Heath in Dorset. The Druce Herbarium at Oxford has two sheets from Surrey and two from Heath, Bucks, of specimens identified by W. H. Beeby as of the "hybrid between *J. lamprocarpus* and *J. acutiflorus*". The identifications were confirmed by Fr. Buchenau, and the plants are undoubtedly hybrids with proliferated inflorescences as found by us at Wytham and Hinksey, Berks, and at Weston, Oxfordshire.

In his monograph on Juncaceae in *Das Pflanzenreich*, Buchenau (1906) records the hybrid as having been identified at several localities in Germany and by Beeby in Surrey. It is of interest that Wulff (1938) gives 60 as the diploid number of

chromosomes for *J. articulatus*, this being the record included in "The Merton Catalogue" (Maude, 1939). Whilst it is possible that there is a fertile race of jointed rushes with 60 chromosomes, it seems most probable that Wulff's counts were made on hybrids.

It is evident from these records that the hybrid between *J. articulatus* and *J. sylvaticus* is very widespread, and that under certain favourable conditions it may be the most abundant jointed rush over wide areas. These conditions are found

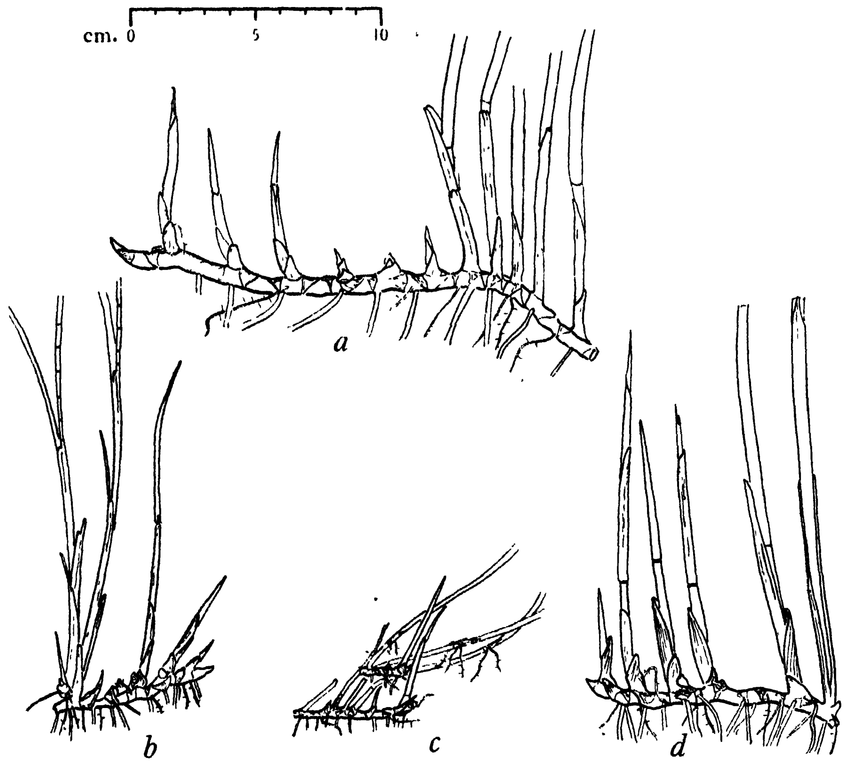

Fig. 4. Drawings of rhizomes of the four described types. *a*, *J. sylvaticus*. *b*, *J. sylvaticus* × *J. articulatus*. *c*, *J. articulatus*. *d*, "Large 80". In *c* an axillary bud near the base of last year's flowering shoot has given rise to a new rhizome at a higher level than the parent rhizome. The flowering shoots of this plant were over 60 cm. long, but were decumbent. The slender rhizome contrasts strikingly with that of *d*, whose flowering shoots were about the same length, but erect.

in the extensive alluvial meadows near Oxford and especially in those damper meadows from which grazing animals are excluded until the hay crop is cut in July or in August.

The status and synonymy of "large 80"

The status of "large 80" is still uncertain. While having the chromosome number of *J. articulatus*, it shows in its vegetative and reproductive morphology a mixture of the characters of *J. articulatus* and *J. sylvaticus*. It resembles *J. sylvaticus*

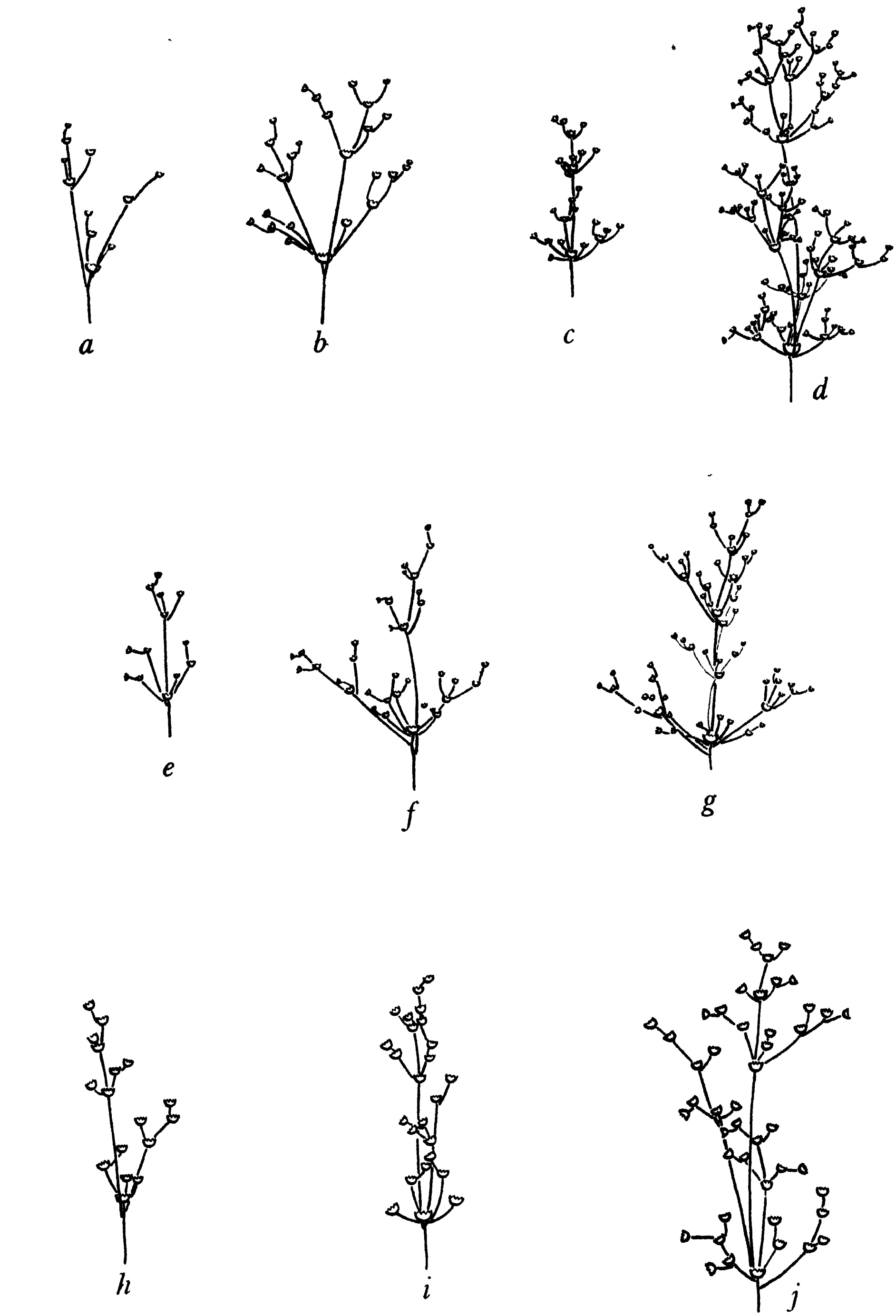

Fig 5. Diagrams showing the architecture of the inflorescences of the four types. $\times \frac{1}{3}$. *a*, *b*, *J. articulatus*. *c*, *d*, *J. sylvaticus*. *e*, *f*, *g*, *J. sylvaticus* × *J. articulatus*. *h*, *i*, *j*, "Large 80".

vegetatively in the strong creeping rhizome, the sturdy erect shoots, the small number of leaves, the length of the leaves, and the easy visibility of their articulations. In the architecture of its inflorescence it also resembles *J. sylvaticus* in that there is a differentiation of the branches into steeply ascending main branches and divaricating shorter branches. The number of flowers per head is large (14–18), and so nearer that in *J. sylvaticus* (7–11) than that in *J. articulatus* (4–8). In the individual flowers, both sets of perianth segments are acute or even mucronate, the

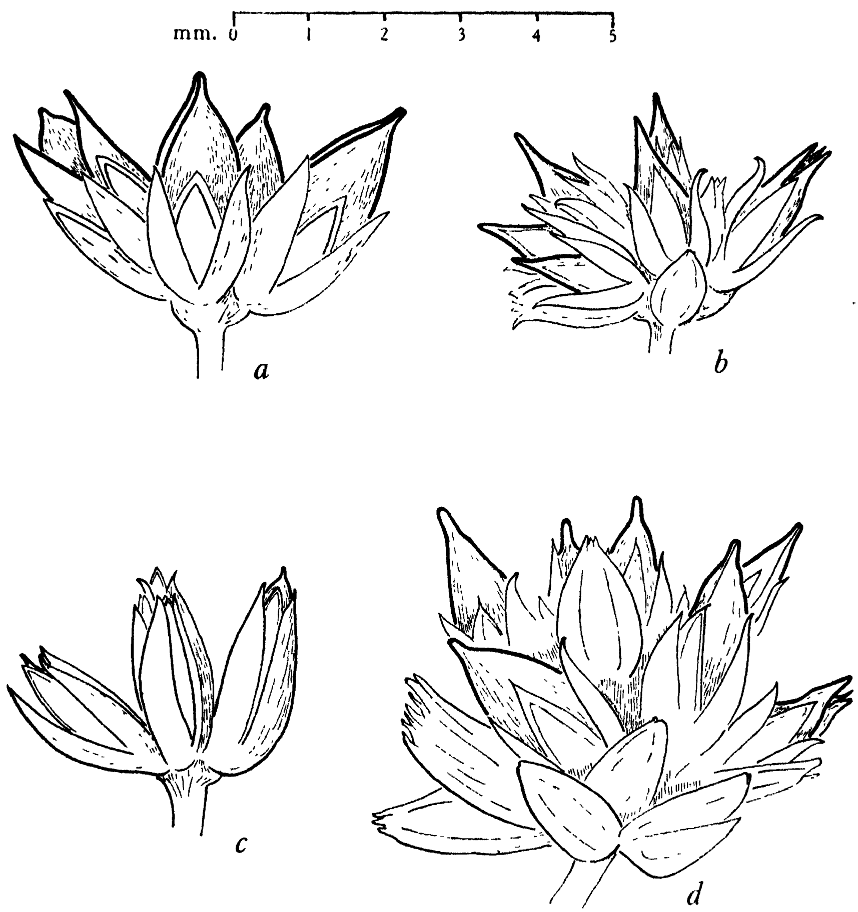

Fig. 6. Camera lucida drawings of typical heads of flowers. *a*, *J. articulatus*. *b*, *J. sylvaticus* *c*, *J. sylvaticus* × *J. articulatus*. *d*, "Large 80".

inner being longer than the outer which are often recurved, as in *J. sylvaticus*. The style is long, and the anthers are longer than their filaments. The capsule is a chestnut brown colour at maturity, not black as in *J. articulatus*, and it is narrowly acuminate. Characters in which there is more similarity to *J. articulatus* are the greater breadth and flattening of the leaves than in *J. sylvaticus*, the size of the inflorescence (though not its architecture), the small number of heads and the considerable length of their peduncles, the large size of the flowers, the broad transparent margin of the inner

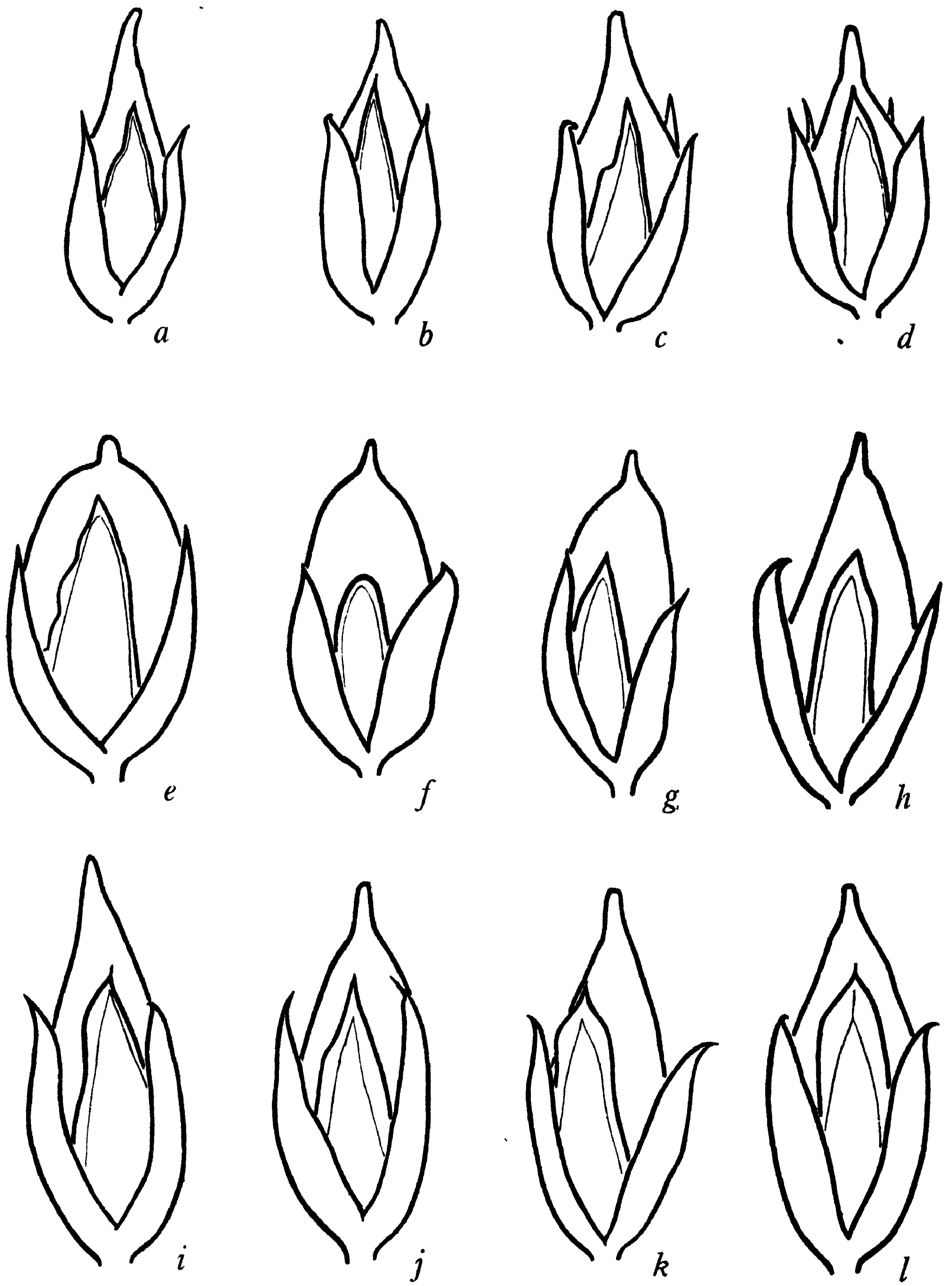

Fig. 7. Camera lucida drawings of typical capsules. × 12. *a*, *b*, *c*, *d*, *J. sylvaticus*. *e*, *f*, *g*, *h*, *J. articulatus*. *i*, *j*, *k*, *l*, "Large 80".

perianth segments, the large size of the capsule and the glossiness of the ripe capsule.

There are three main possibilities as to the taxonomic status of this type. First, it may be merely a variety of *J. articulatus*, with which it agrees in chromosome number and in the taxonomically important feature of a shining and deeply coloured fruit. Examination of the herbarium material available at Oxford has revealed no sheets which can be referred to "large 80", nor has any description of it as a British plant been so far discovered. Buchenau (1906) lists a variety *cuspidatus* M.Br. whose characters are given as "*Altus. Tepala fere aristato-acuminata; fructus sensim acuminatus. Caet. ut in var. genuino*". It is said to be widely but sparsely distributed, and to be most frequent in Finland and Russia, though it occurs also in Sardinia, and in allied form on Borkum. Reference to the original description by Brenner (1889) elicits only the further information that students of the Finnish flora have often confused this variety with *J. sylvaticus*, which does not appear to occur on the Finnish mainland. While these descriptions agree in some respects with "large 80", it is by no means certain that we have Brenner's variety. Thus in Buchenau's key it is placed in the subsection with "heads small, 4–10 flowers", but "large 80" usually has 14–18 flowers per head. Nor is any mention made of the strikingly vigorous rhizome and the stiffly erect aerial shoot, so different from those of *J. articulatus*. Moreover, no transitional forms were found between "large 80" and typical *J. articulatus* actually growing with it. This suggests that even if it has already been described as var. *cuspidatus* M.Br. there are bars to intercrossing with typical *J. articulatus* and that its taxonomic status needs reconsideration.

The second possibility is that "large 80" is a tetraploid derivative of *J. sylvaticus*. The evidence in favour of this view, apart from the several features of similarity to *J. sylvaticus*, are the robustness of the plants, and the facts that the stomata and seeds are larger than in *J. sylvaticus* or in typical *J. articulatus*, and that the number of seeds is comparatively small (*c.* 19) in view of the large size of the capsule. The capsules of *J. sylvaticus* are very much smaller, yet counts on Oxford material gave an average of 12·4 seeds per capsule, and as many as 21 have been found. *J. articulatus* has capsules not very different in volume from those of "large 80", but the average number of seeds found was 41·4. This suggests a reduced fertility as compared with either species. The crucial observations on multivalent formation and unequal disjunction at meiosis could not be made, owing to failure to fix flower buds at the right stages.

The last possibility is that "large 80" has a complex history, as the result of which it really does combine genetic material from *J. articulatus* and *J. sylvaticus*. It might, for instance, be the product of the fusion of an unreduced *sylvaticus* and a normal *articulatus* gamete, of the latter with a viable gamete produced by the hybrid and having 40 chromosomes, or of two such gametes from the hybrid. There are evidently many other such ways in which a fertile type with 80 chromosomes might arise.

It is clear that further investigations are required before any well-founded conclusions can be drawn concerning the status of "large 80", and it is proposed

to continue this part of the work next season by studying meiosis and the results of selfing and of crossing with *J. sylvaticus* and *J. articulatus*.

SUMMARY

Cytological and morphological investigations on the jointed rushes of the Oxford district show that the sterile hybrid between *Juncus articulatus* L. and *J. sylvaticus* is a very common plant near Oxford. The hybrid and the two parent species are described and compared, and it is shown that the hybrid is widely distributed in Britain. A jointed rush having the same chromosome number as *J. articulatus* but differing from it in many respects is also described under the designation "large 80". Its taxonomic status is not yet known.

In conclusion, we wish to express our gratitude to Mr H. Baker of the Department of Botany, whose intimate knowledge of the flora of the Oxford district has been placed most generously at our disposal.

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APPENDIX

Detailed record of plants on which chromosome counts were taken
(counts on root tips unless otherwise stated)

Designation	Notes	Chromosome no.
	1. WYTHAM, BERKS	
	(*a*) *From the seepage zone south of Hagley Pool*	
T 2, T 3, W 1, W 7	Typical *J. articulatus*	80
J.A.B.	Collected in late autumn	60
W 5, W 6	Slender plants, less rigid than W 2–W 4, with shorter leaves; creeping rhizomes	60
W 2, W 3, W 3[1], W 4, Z 1*a*, Z 1*b*	Erect sturdy plants, variable in height with strong creeping rhizomes: typical "large 80"	80
	(*b*) *From the lower meadow south of Hagley Pool*	
V 18	Typical *J. articulatus*: counts on pollen grains, mitotic plates	80
	(*c*) *From the mowing meadow north of Hagley Pool*	
M 1, M 2	Collected in winter from depressions near the river	80
W 11	Large decumbent plant growing on the path near the Pool: typical *J. articulatus*	80
T 1, W 8, W 9, W 10	Slender ascending plants with a small number (*c.* 3) of long, slender, shining leaves: apparently sterile, but taken shortly after flowering: typical of the large population just north of Hagley Pool	60

Designation	Notes	Chromosome no.
	2. PIXEY MEAD, OXON.	
E	Vigorous plant collected in winter from the small meadow between Pixey Mead and the Wolvercote Road	40
F	Smaller plant from same locality as E	60
G	Plant from the uniform population of the main mead: typical *J. sylvaticus*	40
	3. OXEY AND YARNTON MEADS, OXON.	
N 1, N 2	At head of drain in Oxey Mead: typical *J. articulatus*	80
R 1, R 2, R 3, R 4, R 5, R 6, R 7, R 8, R 9	Prostrate and ascending plants from the main path and near it in the narrow neck connecting Oxey and Yarnton Meads: sterile	60
S 1, S 2, S 3, S 4, S 5	From higher and drier ground towards the west end of Yarnton Mead: typical *J. sylvaticus*	40
S 6, S 7	From main path in Yarnton Mead: prostrate plants	60
	4. PORT MEADOW, OXON.	
O 1, O 2, O 3	From large patch of small, semi-prostrate and stoloniferous plants with 5–7 leaves per shoot at east edge of the Meadow	80
P	Similar plants close to main stream opposite Medley	80
	5. GODSTOW, OXON.	
T 5, T 6	Large decumbent plants on bank of main stream, with some shoots floating: leaves very short, broad and deeply striate; strikingly vigorous *J. articulatus*	80
	6. IFFLEY MEADOW, BERKS	
O 1, O 2, O 3	From west edge of the meadow south-west of Iffley Lock: sterile	60
Y 4	Single small decumbent plant found nearer to the main stream	80
	7. BAGLEY WOOD, BERKS	
J.A.L.	Tall erect plant from a small population at seepage line where Plateau Gravel overlies Kimmeridge Clay: typical *J. sylvaticus*	40
	8. COTHILL, BERKS	
J.A.D.	Small decumbent plants from pathway on deep peat in Morland's Meadow	80
J.S.A., J.O.A.[2]	Strong, erect plants from grass verge of String Lane	40
J.A.A.	Smaller trampled plant from String Lane	40
	9. MARCHAM, BERKS	
J.S.D. 1, J.S.D. 2, J.S.D. 3	Typical *J. sylvaticus* from a lightly grazed and occasionally mown meadow on peaty soil	40

Designation	Notes	Chromosome no.
	10. WESTON, OXON.	
X 1, X 2, X 6	Typical *J. articulatus* from stream side	80
X 3, X 4, X 5	Tall, slender, ascending plants from bed of streamlet: sterile	60
	11. MATLEY BOG, HANTS	
J.S.C., J 2, J 3, K 1, K 2, K 4	Samples from main populations north and south of the stream: typical *J. sylvaticus*	40
J 1, Y 3	Smaller ascending plants: Y 3 had *J. articulatus*-like capsules, but empty: leaves more terete and longer than in *J. articulatus*	60
	12. GREENHAM COMMON, BERKS	
J.S.B.	Typical *J. sylvaticus* from valley bog	40
	13. HOLT LOWS, NORFOLK	
J.a.C.	Typical *J. sylvaticus* from valley bog	40
	14. GLENMORE, EAST INVERNESS	
Y 1	Small ascending plant, with shining leaves; "from the grassy side of a watercourse under pine trees" (E. F. Warburg)	60
	15. LOCH-AN-EILAN, EAST INVERNESS	
Y 2	Small, erect plants from gravelly shore of the Loch	80

CHROMOSOME NUMBERS IN SOME BRITISH PLANTS

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(With 10 figures in the text)

1. Introduction

While preparing the "Merton Catalogue" of chromosome numbers of British flowering plants I made a number of new chromosome counts. The object was partly to help complete this survey and partly to find, if possible, new types of variation in chromosome number within a species. In cases where variation was suspected or discovered, as in *Arum*, plants from several different localities were looked at, but in most cases only one was examined. Plants from a number of different families were chosen, mostly because they were common and easily obtained, or because they belonged to genera whose basic number was unknown, or to those of which no British species had been counted. The Gramineae was the only family where a large number of species (24) was examined.

2. Material and methods

All the material used was of British origin with the exception of *Veronica alpina*, which was kindly brought to me from Iceland by Dr N. Polunin of Oxford, and *Scilla pratensis*, which is not a British species. I am very grateful to Mr E. M. Marsden-Jones of the Potterne Biological Station for supplying the greater part of my material from his collection, and for advice on several points. My thanks are also due to the staff of the Royal Botanic Gardens, Kew, who sent me seed and plants, and particularly to Mr C. E. Hubbard for much of my grass material and for making my wants known to several botanists who were able to send me plants from different parts of England. Finally, I have to thank my colleagues at the John Innes Horticultural Institution who brought me plants from the wild.

Except for some of the grasses, I have used the name given in the *London Catalogue of British Plants* (11th ed.).

In all cases except where there is a note to the contrary, the chromosome number was determined in mitotic divisions in root tips. These were fixed in 2 BE (La Cour, 1937) and stained by Newton's gentian violet-iodine method.

Sections were cut at thicknesses varying from 8μ in the case of the Rosaceae to 24μ in the case of *Allium*. 12μ is a suitable thickness for most dicotyledons, while for the grasses a thickness of 15–20μ is necessary.

3. Chromosome numbers

(*a*) The following are new counts in British species:

Dicotyledons

	2n	Fig.		2n	Fig.
Clematis Vitalba	16	1 (*a*)	*Anaphalis margaritacea*	28	2 (*k*)
Epimedium alpinum	12	1 (*d*)	*Petasites fragrans*	52	2 (*h*)
Corydalis bulbosa	24	1 (*g*)	*Serratula tinctoria*	22	2 (*i*)
Diplotaxis muralis	42	1 (*f*)	*Arnoseris minima*	18	2 (*j*)
Ilex Aquifolium	40	1 (*e*)	*Erica vagans*	24	3 (*a*)
Lupinus nootkatensis	48	1 (*h*)	*Dabeocia cantabrica*	24	3 (*b*)
Genista anglica	42	1 (*k*)	*Anagallis tenella*	22	3 (*d*)
Cytisus scoparius	46	1 (*i*)	*Blackstonia perfoliata*	44	3 (*c*)
Ornithopus perpusillus	14	1 (*l*)	*Symphytum peregrinum*	36	3 (*e*)
Hippocrepis comosa	28	1 (*j*)	*Scrophularia aquatica*	80	3 (*i*)
Poterium Sanguisorba	28	2 (*c*)	*Mimulus Langsdorffii*	48	3 (*h*)
Dryas octopetala	18	2 (*d*)	*Veronica alpina*	18	3 (*f*)
Silaus flavescens	22	2 (*e*)	*Ajuga reptans*	32	3 (*k*)
Heracleum Sphondylium	22	2 (*f*)	*Salicornia perennis*	18	4 (*e*)

Monocotyledons

	2n	Fig.		2n	Fig.
Sisyrinchium californicum	34	4 (*f*)	*Koeleria vallesiana*	42	8 (*c*)
Allium sibiricum	16	5 (*d*)	*K. gracilis*	28 (30)	8 (*a*, *b*)
A. Ampeloprasum	32	5 (*c*)	*Poa Balfourii*	42	8 (*e*)
A. Babingtonii	48	5 (*a*, *b*)	*Glyceria declinata*	20	9 (*c*)
Scilla verna	22	6 (*a*)	*Puccinellia maritima*	63	9 (*a*)
S. autumnalis	44 ?		*Festuca rigida*	14	10 (*d*)
Alopecurus bulbosus	14	7 (*a*)	*F. ambigua*	28	10 (*e*)
Agrostis setacea	14	7 (*b*)	*F. fallax*	42	10 (*c*)
A. nigra	42	7 (*c*)	*F. longifolia*	42	10 (*a*)
Apera interrupta	14	7 (*g*)	*F. uniglumis*	42	10 (*b*)
Aira praecox	14	7 (*e*)	*Bromus interruptus*	28	9 (*g*)
Deschampsia setacea	14	7 (*f*)	*B. lepidus*	28	9 (*f*)
Avena pratensis	42	8 (*d*)	*B. racemosus*	28	9 (*e*)
Sieglingia decumbens	124	7 (*d*)			

(*b*) The following counts confirm those of earlier authors:

	2n	Fig.	Author
Agrimonia Eupatoria	28	2 (*b*)	Wulff 1938
Daucus Carota	18		Lindenbein 1932
Matricaria Chamomilla	18	2 (*g*)	Beer 1912
Anchusa sempervirens	22	3 (*g*)	Smith 1932
Chenopodium album	18 (36, 54)	4 (*a*, *b*, *c*)	Winge 1917
Suaeda maritima	36		Wulff 1937
Hydrocharis Morsus-ranae	28	6 (*c*)	Tushnajakowa 1929
Daphne Mezereum	18	3 (*j*)	Strasburger 1909
Ruscus aculeatus	40	6 (*d*, *e*)	Nakajima 1936
Deschampsia alpina	49		Flovik 1938

(*c*) The following counts differ from those of earlier authors:

	2n	Fig.	Author	2n
Meconopsis cambrica	22	1 (*b*)	Sugiura 1937	28
Spiraea Filipendula	15	2 (*a*)	Wulff 1938	14
Daucus Carota	18		Melderis 1930	22
Anchusa sempervirens	22	3 (*g*)	Sugiura 1937	16
Salicornia herbacea	36 + 2*ff*	4 (*d*)	Wulff 1937	38
Arum maculatum	56, 84	4 (*g*, *h*)	Schmucker 1925	32
Glyceria fluitans	40	9 (*b*)	Stählin 1929	28
G. plicata	40	9 (*d*)	Stählin 1929	28

(*d*) One new non-British species has been counted:

	2n	Fig.
Scilla pratensis	28	6 (*b*)

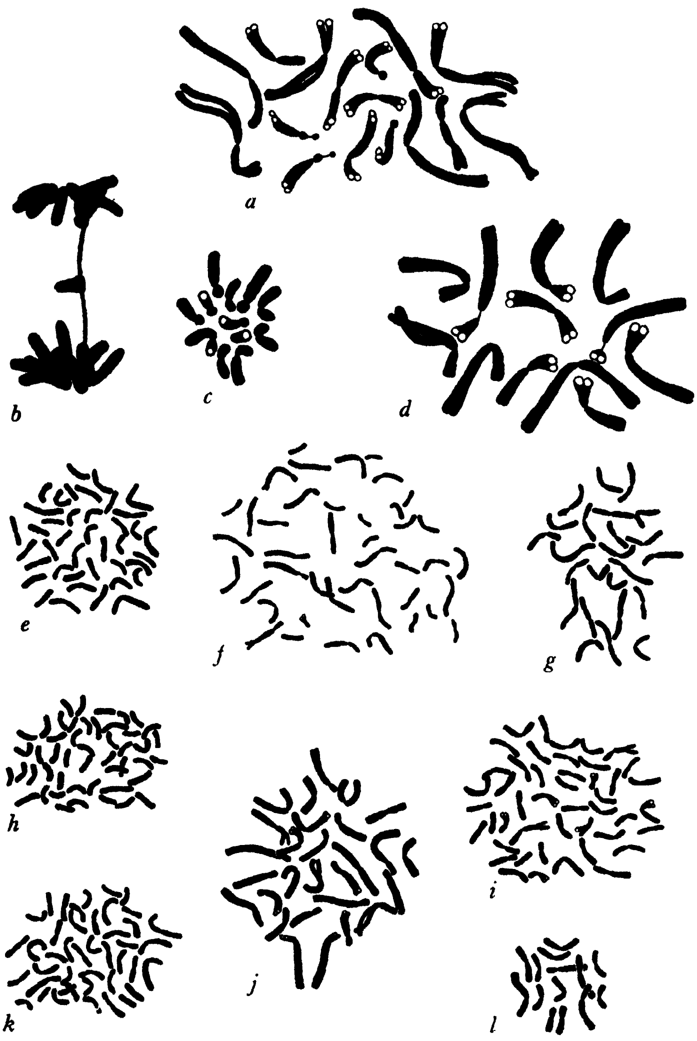

Fig. 1. *a*, *Clematis Vitalba*, $2n=16$; *b*, *Meconopsis cambrica*, pollen-grain division; *c*, same, $n=11$ (pollen-grain mitosis); *d*, *Epimedium alpinum*, $2n=12$; *e*, *Ilex Aquifolium*, $2n=40$; *f*, *Diplotaxis muralis*, $2n=42$; *g*, *Corydalis bulbosa*, $2n=24$; *h*, *Lupinus nootkatensis*, $2n=48$; *i*, *Cytisus scoparius*, $2n=46$; *j*, *Hippocrepis comosa*, $2n=28$; *k*, *Genista anglica*, $2n=42$; *l*, *Ornithopus perpusillus*, $2n=14$. All drawings are $\times$ 19c o

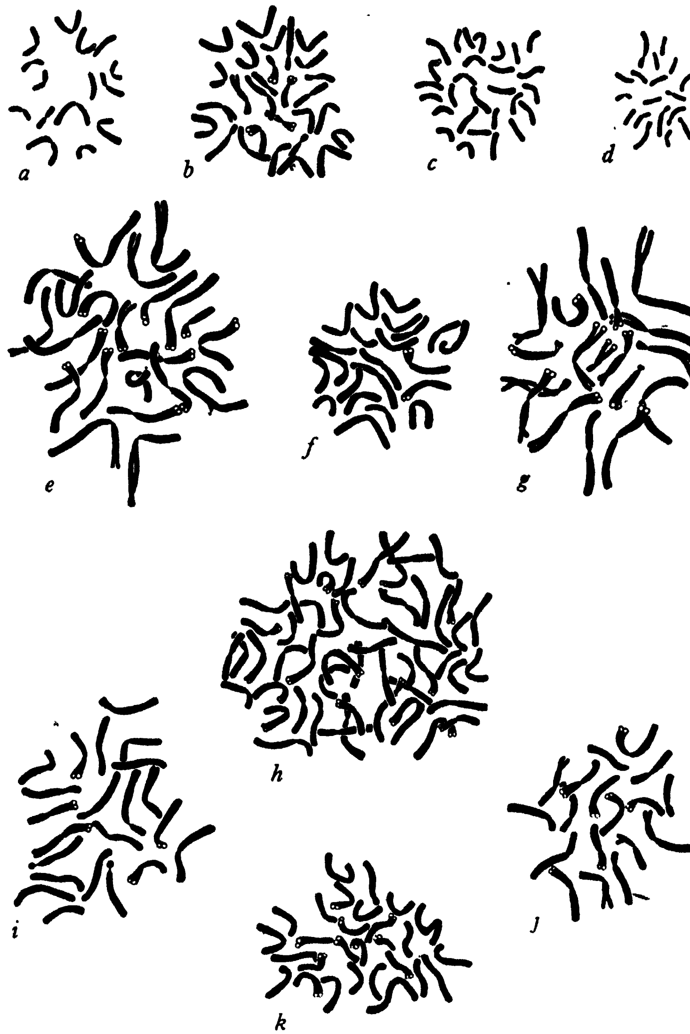

Fig. 2. *a*, *Spiraea Filipendula*, $2n = 15$; *b*, *Agrimonia Eupatoria*, $2n = 28$; *c*, *Poterium Sanguisorba*, $2n = 28$; *d*, *Dryas octopetala*, $2n = 18$; *e*, *Silaus flavescens*, $2n = 22$; *f*, *Heracleum Sphondylium*, $2n = 22$; *g*, *Matricaria Chamomilla*, $2n = 18$; *h*, *Petasites fragrans*, $2n = 52$; *i*, *Serratula tinctoria*, $2n = 22$; *j*, *Arnoseris minima*, $2n = 18$; *k*, *Anaphalis margaritacea*, $2n = 28$.

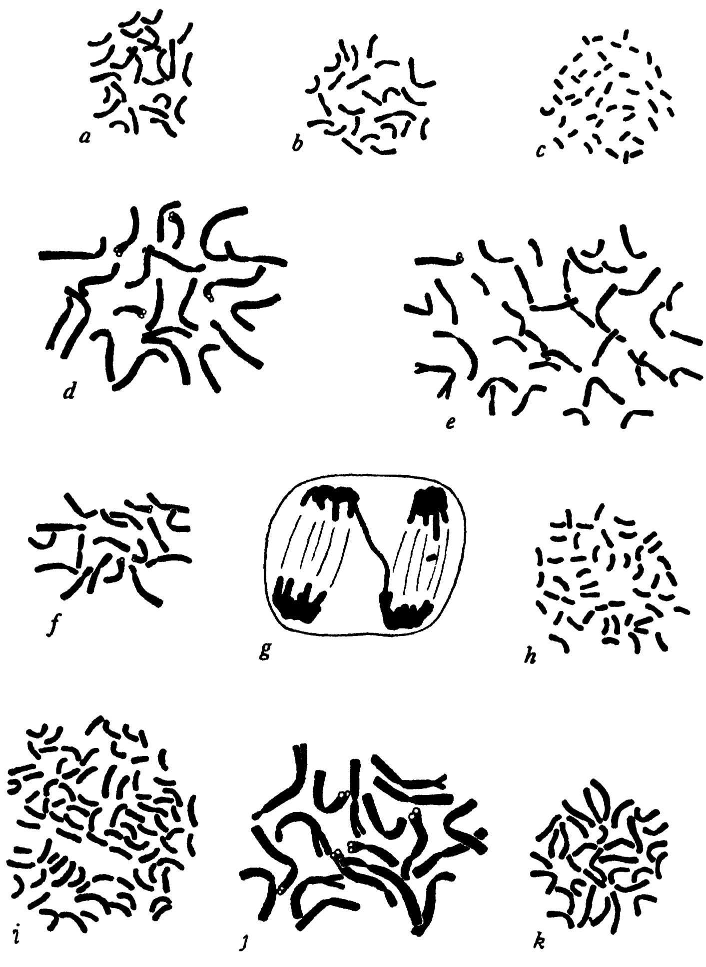

Fig. 3. *a*, *Erica vagans*, $2n=24$; *b*, *Dabeocia cantabrica*, $2n=24$; *c*, *Blackstonia perfoliata*, $2n=44$; *d*, *Anagallis tenella*, $2n=22$; *e*, *Symphytum peregrinum*, $2n=36$; *f*, *Veronica alpina*, $2n=18$; *g*, *Anchusa sempervirens*, pollen mother cell division; *h*, *Mimulus Langsdorffii*, $2n=48$; *i*, *Scrophularia aquatica*, $2n=80$; *j*, *Daphne Mezereum*, $2n=18$; *k*, *Ajuga reptans*, $2n=32$.

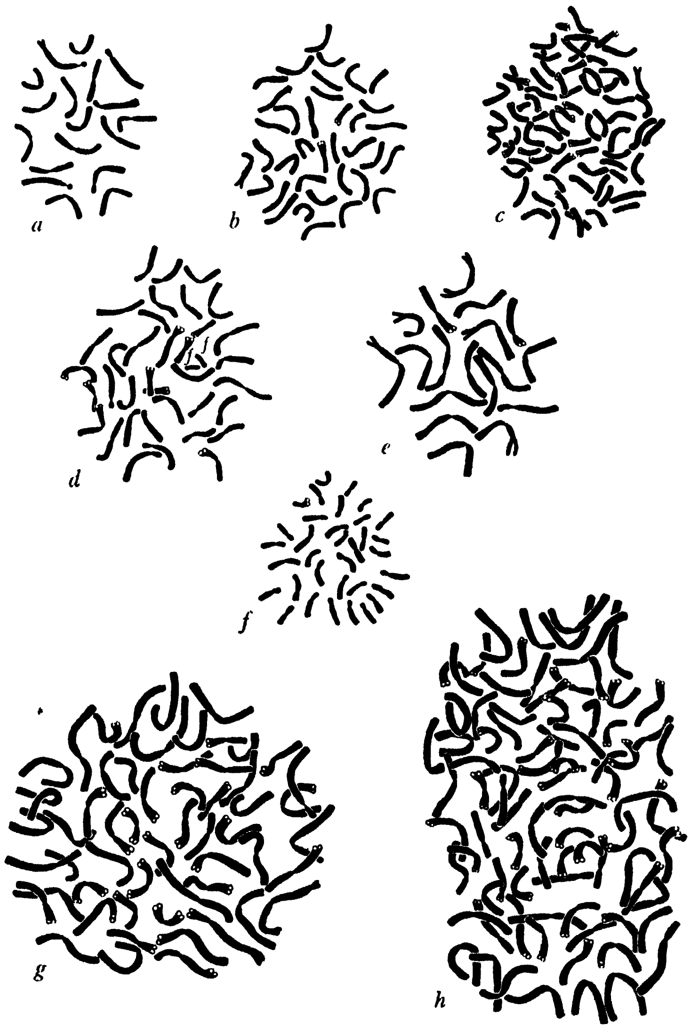

Fig. 4. *a*, *Chenopodium album*, $2n = 18$ ($2x$); *b*, the same, $2n = 36$ ($4x$); *c*, the same, $2n = 72$ ($8x$); *d*, *Salicornia herbacea*, $2n = 36 + 2ff$; *e*, *Salicornia perennis*, $2n = 18$; *f*, *Sisyrinchium californicum*, $2n = 34$; *g*, *Arum maculatum*, $2n = 56$; *h*, the same, $2n = 84$.

Fig. 5. *a*, *Allium Babingtonii*, $2n=48$; *b*, single chromosome of the same, from longitudinal section of metaphase plate; *c*, *A. Ampeloprasum*, $2n=32$; *d*, *A. sibiricum*, $2n=16$.

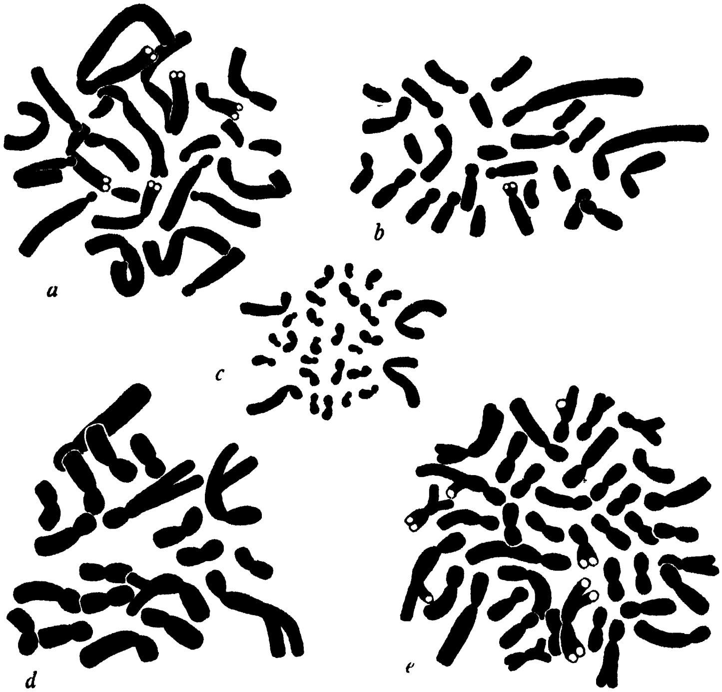

Fig. 6. *a*, *Scilla verna*, $2n = 22$; *b*, *S. pratensis*, $2n = 28$; *c*, *Hydrocharis Morsus-ranae*, $2n = 28$ (drawn from a slide made by Miss Hoare, Royal Holloway College); *d*, *Ruscus aculeatus*, $n = 20$ (pollen-grain division, stained with aceto-carmine); *e*, root-tip, $2n = 40$ (stained with gentian violet).

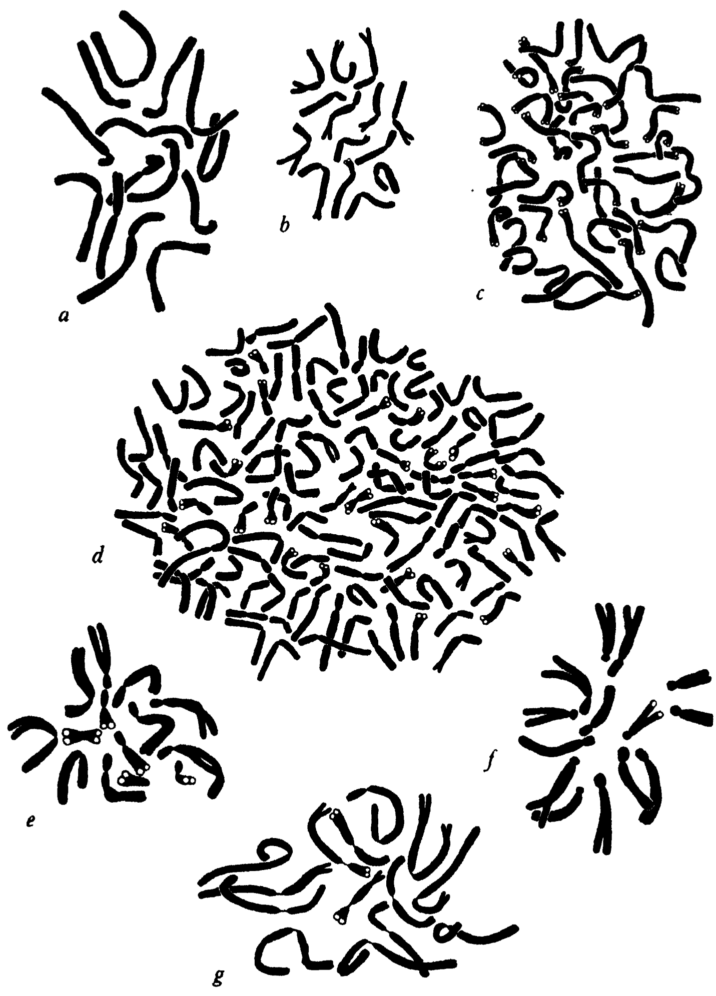

Fig. 7. *a*, *Alopecurus bulbosus*, $2n=14$; *b*, *Agrostis setacea*, $2n=14$; *c*, *A. nigra*, $2n=42$; *d*, *Sieglingia decumbens*, $2n=124$; *e*, *Aira praecox*, $2n=14$; *f*, *Deschampsia setacea*, $2n=14$; *g*, *Apera interrupta*, $2n=14$.

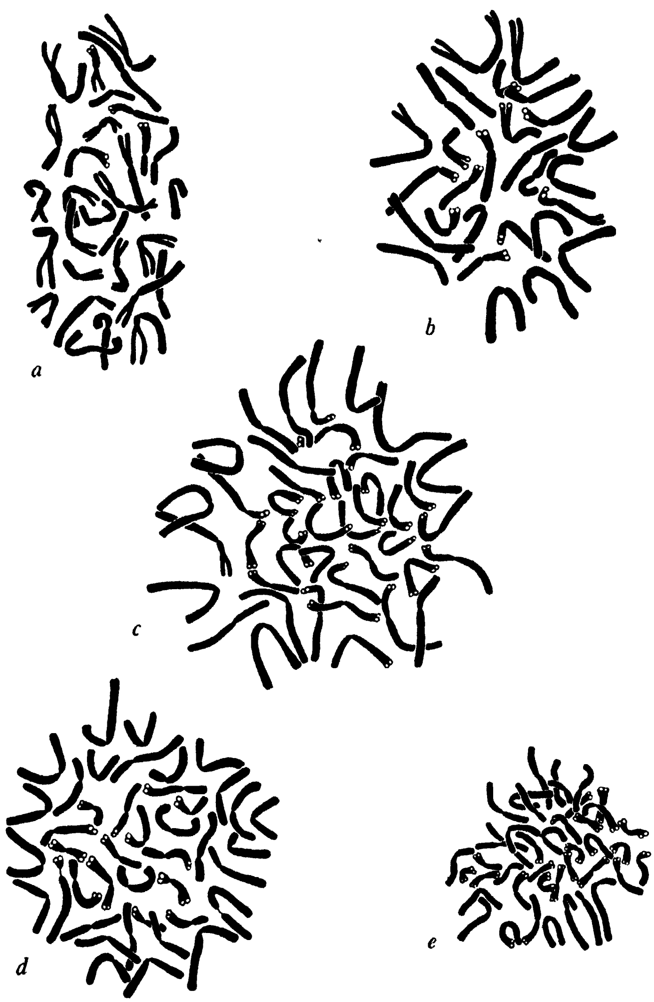

Fig. 8. *a*, *Koeleria gracilis*, $2n=30$; *b*, the same, $2n=28$; *c*, *K. vallesiana*, $2n=42$; *d*, *Avena pratensis*, $2n=42$; *e*, *Poa Balfourii*, $2n=42$.

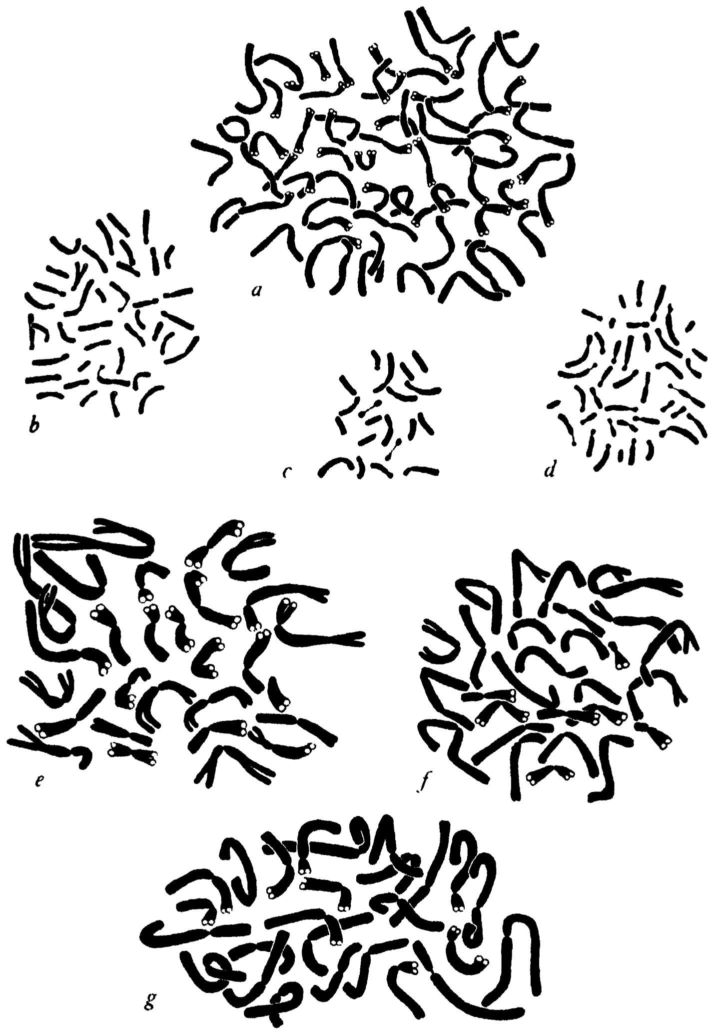

Fig. 9. *a*, *Puccinellia maritima*, $2n=63$; *b*, *Glyceria fluitans*, $2n=40$; *c*, *G. declinata*, $2n=20$; *d*, *G. plicata*, $2n=40$; *e*, *Bromus racemosus*, $2n=28$; *f*, *B. lepidus*, $2n=28$; *g*, *B. interruptus*, $2n=28$.

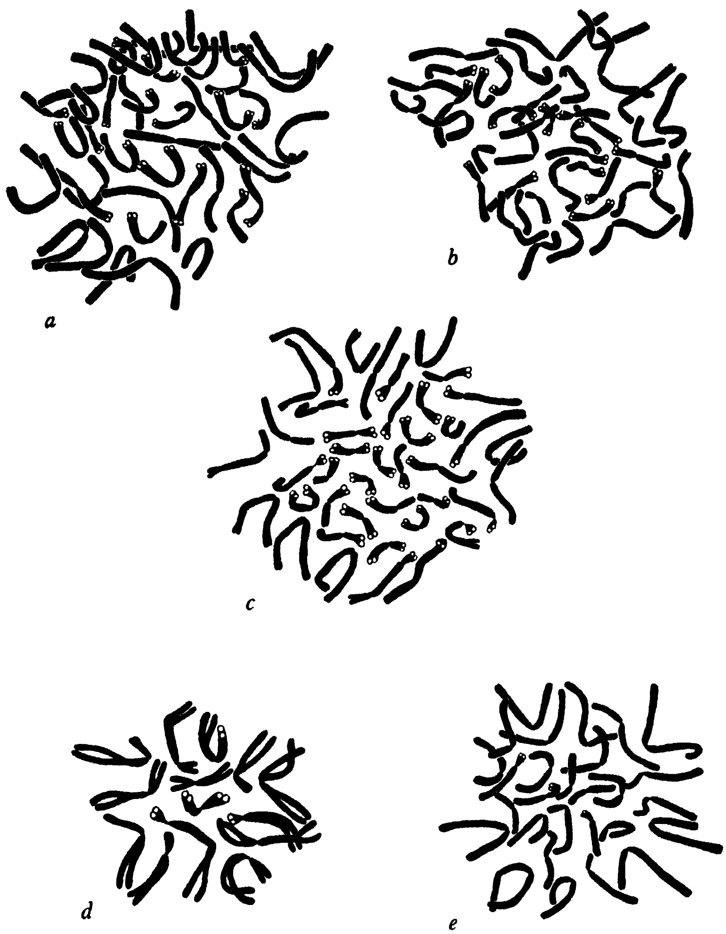

Fig. 10. *a*, *Festuca longifolia*, $2n = 42$; *b*, *F. uniglumis*, $2n = 42$; *c*, *F. fallax*, $2n = 42$; *d*, *F. rigida*, $2n = 14$; *e*, *F. ambigua*, $2n = 28$.

4. General observations

A. *Chromosome size*

The relation between chromosome size and cell size varies rather little in most species, as can be seen by comparing the cell plates in the figures. There are some species, however, with chromosomes much more widely-spaced than the normal: this is the case in *Diplotaxis muralis* ($2n=42$, Fig. 1*f*) and in *Symphytum peregrinum* ($2n=36$, Fig. 3*e*).

In most plants the length of the chromosomes within the complement is fairly uniform, though it is not uncommon for there to be a few pairs a good deal shorter than the standard length. A very much rarer condition is found in *Scilla pratensis*, where one pair of chromosomes is more than twice as long as the next longest pair (Fig. 6*b*) the lengths being 6·7 and 3μ. The same condition occurs in *Hydrocharis Morsus-ranae* (Fig. 6*c*), where two pairs of chromosomes are very much longer than the rest, and to a lesser extent in *Crocus cancellatus* (La Cour, unpublished). Occasionally the sex chromosomes are much longer than the others in dioecious plants and animals.

B. *Variations of chromosome number within the species*

Several species have been found to have variations in the chromosome number. This is probably much commoner than is at present known, and new cases will be discovered as specimens of a species from different localities are examined.

One plant of *Koeleria gracilis* ($2n=28$) was found with 30 chromosomes (Figs. 8*a*, *b*).

One of the most usual causes of variation in chromosome number is polyploidy. This accounts for the different numbers in *Chenopodium album*, which in Sweden has been found to have $2n=18$ (Winge, 1917) and $2n=54$ (Kjellmark, 1934), and in America $2n=36$ (Cooper, 1935). My material, found as a weed in the grounds of the John Innes Institution, had $2n=18$. It is possible that there are also tetraploids and hexaploids in Britain, and that they account partly for the great variability of the species.

The occurrence of somatic doubling in cells of the roots is well known in the Chenopodiaceae, and in *C. album* I found diploid (18), tetraploid (36) and octoploid (72) cells in the same root tip. The plates with 36 and 72 chromosomes were more crowded and the chromosome ends more turned up than those with 18, so that the chromosomes appear shorter and the trabants cannot be seen (Fig. 4*a–c*). The other authors of chromosome counts in *C. album* did not look at the root tips, so that it is not known whether doubled cells are found in the polyploid varieties as well. Meurman (1933) found a triploid seedling of *Acer platanoides* with hexaploid cells, but possibly in a higher polyploid such as the hexaploid *Chenopodium album* there might be no somatic doubling.

Another species with a variety of chromosome numbers that are probably to be accounted for by polyploidy is *Arum maculatum*. Schmucker (1925) gives the

number as $2n=32$, while I have found $2n=56$ in plants from Cheshire, Devonshire, Sussex and the Rhineland (Germany), and $2n=84$ in several plants from Merton (Surrey) and Oxted (Surrey). *A. italicum*, a species differing from *A. maculatum* chiefly in its greater size, is said to have $2n=64$ (Dangeard, 1937). Assuming the basic number to be 8, the plant with $2n=32$ would be tetraploid, while mine with $2n=56$ are heptaploid, and the ones with $2n=ca.$ 84 have ten or eleven sets of chromosomes. Where polyploidy is so high, chromosomes can probably be gained or lost without disturbing the balance. It is curious that in all my material there has been no tetraploid plant. Alternatively the basic number might be 7, and Schmucker's count should have been $2n=28$.

A. maculatum is a plant which shows very marked morphological variations, the most striking being in the pigmentation. The leaves may be spotted with purple or white, the spots varying greatly in size and number. The spathe may also be spotted, and may have a purple edge. The spadix varies in colour from yellow to deep purple, as do the anthers, and there are other easily recognizable variations. Differences in leaf size are impossible to estimate without knowing the age of the plant, as the leaves do not reach full size in the first few years. The plants which had 84 chromosomes did not seem to be abnormally large, but I did find one group of giant plants, growing in a wood on the chalk in Surrey (Hog Trough Lane above Barrow Green, Oxted) with leaves measuring about 28×14 cm. as against the 16×7 cm. which is the normal size for a full-grown leaf. Unfortunately I was unable to count the chromosomes of these plants, and I did not see the flowers. The leaves were dark green spotted with purple. *A. italicum*, which is said to grow in Sussex, has paler green leaves with white veins, so the giant plant cannot have belonged to this species.

Certain counts, although showing no variation in chromosome number themselves, nevertheless imply it. Such are triploids, since they nearly always have close diploid relatives. A possible triploid is *Corydalis bulbosa* ($2n=24$). Of the three species of *Corydalis* previously counted, two have $2n=16$ and the third has $2n=56$. It is possible that the basic number of 8 holds for *C. bulbosa*, in which case it would be a triploid. It reproduces vegetatively by bulbs and also sets seed, which would presumably be apomictic.

C. *Higher polyploids and apomixis*

Allium Babingtonii, with the diploid chromosome number of 48, is presumably a hexaploid. The only other *Allium* with as many chromosomes is *A. roseum* var. *bulbilliferum* (Messeri, 1931) and both species reproduce in nature by bulbils. In many plants polyploidy, particularly autopolyploidy, is associated with a greater importance of vegetative reproduction, e.g. vivipary in *Festuca ovina* (Turesson, 1930, 1931), bulbil-formation in *Ranunculus Ficaria* (Larter, 1932), stolons in *Tulipa* and bulbils in *Lilium* (see Levan, 1937). The chromosomes of *Allium Babingtonii* are among the largest known, the longest one drawn being 23μ. None of the species measured by Levan (1935) had chromosomes longer than 15μ, except *Nothoscordum* (*Allium*) *fragrans* (22μ).

Sieglingia decumbens (*Triodea decumbens*) ($2=124$, Fig. 7*d*) has the highest chromosome number known in the grasses, except for the recent hybrid *Spartina Townsendii* ($2n=126$). Viable seeds are produced, but the flowers are described as cleistogamous (i.e. fertilization takes place within the closed flower). Beddows (1931) found that anthers were difficult to obtain and that there were only indefinite hair-like stigmas. This combined with the very high chromosome number makes it seem probable that the species is apomictic.

Another species which is probably apomictic is *Puccinellia maritima* ($2n=63$). The basic number is most likely to be 7, giving this species nine sets of chromosomes. *P. maritima* was formerly included in *Glyceria*, which has a basic number of 10, and the separation is justified on cytological grounds. The chromosomes are much larger than those of *Glyceria* (Fig. 9*a–d*).

D. Spiraea Filipendula

Root-tip divisions have been examined in the following plants, and in every case there appeared to be 15 chromosomes as the diploid number:

(1) A double-flowered garden variety from Surrey. These plants produce single-flowered seedlings.

(2) Three of these seedlings from the same garden.

(3) A plant from the Chelsea Physic Garden.

(4) A plant from the Potterne Biological Station.

(5) A double-flowered plant growing at the John Innes Institution.

There are two pairs of long chromosomes with median constrictions, three pairs of medium-sized ones, one of which has subterminal constrictions, two pairs of very small ones and one long unpaired chromosome. The five long chromosomes are easily seen.

This uneven diploid number, if confirmed, cannot be due to a fragment, since the extra chromosome is one of the long ones, nor can it be maintained by apomixis, as the single-flowered seedlings from the double-flowered parent show that fertilization has taken place.

The chromosome number of *S. Filipendula* does not conform with the basic number of 9 found by Sax (1936) in eighteen species of *Spiraea*. Neither does that of *S. Ulmaria*, whose number is given as $2n=14$ and $2n=16$ by Wulff (1938) and Turesson (1938), respectively.

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THE MECHANISM OF PHOTOSYNTHESIS IN GREEN PLANTS

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(With 4 figures in the text)

CONTENTS

List of symbols employed

v	Rate of assimilation per second.
Δ	Yield of assimilation per flash.
τ	Blackman period.
t	Interval between arrival of two quanta at a reducing centre.
n	Number of reducing centres.
ν	Number of chlorophyll molecules.
$Z=\nu/n$	Numerical photosynthetic unit.
z	Functional photosynthetic unit.
z/Z	Efficiency of chlorophyll.
N	Number of quanta per second.
$N_{(\frac{1}{2})}$	Number of quanta per second at half maximum rate.
$N_{(\frac{1}{2})\,\mathrm{eff.}}=N_{(\frac{1}{2})}\,z/Z$	Number of effective quanta per second at half maximum rate.
$\mathbf{N}$	Number of quanta per flash.
$\mathbf{N}_{\mathrm{eff.}}=\mathbf{N}\,z/Z$	Effective number of quanta per flash.
ϑ	Photoperiod.

INTRODUCTION

PHOTOSYNTHESIS in green plants means the transformation of carbon dioxide and water into oxygen and carbohydrate by the action of light. The empirical formula of this process is very simple and is written

$$nCO_2 + nH_2O = nO_2 + (CH_2O)n. \qquad (1)$$

The real process, however, by which this result is achieved is quite obscure because we are not acquainted with any of the chemical intermediates nor with any of the catalysts or enzymes engaged in the process. In a sense even one of the final products of photosynthesis is unknown: it is of course known that the product of the reduction of CO_2 has the empirical formula $(CH_2O)n$, yet it is not known whether this product leaves the group of the essentially photosynthetic reactions in the form of formaldehyde or in some more condensed form. In other words it is not clear at what stage of its formation the carbohydrate ceases to have a direct and quasi-stoichiometric influence upon the consumption of CO_2 and the development of O_2, so that its influence on these changes becomes sufficiently faint for it to be considered part of the general biological environment.

Usually the investigation of assimilation is confined to the gas change, i.e. to the velocity of the consumption of CO_2 and the development of O_2, the latter being equal to the former according to equation (1).[1] This "heterogeneous gas reaction" can be influenced of course by chemical means, and such experiments form one of the foundations of our ideas on photosynthesis (cf. e.g. Warburg, 1919, 1920, 1928; Warburg & Uyesugi, 1924; van den Honert, 1930; van der Paauw, 1932; Emerson & Arnold, 1931; Kohn, 1934; Gaffron, 1937, 1939). However, this is not because they have succeeded in identifying any individual chemical substance involved (other than chlorophyll, see below), but chiefly because they give information about the chemical and physical type of the photosynthetic reaction. Thus chemical studies yield information of the same general sort as that provided by the investigation of the effects of variation of other external factors, and so assist in developing a kinetic model of photosynthesis.

The external factors mentioned are CO_2 concentration, temperature, some particular conditions, salt concentrations and pH, cf. e.g. Emerson & Green (1938) and finally light, which far more than in any other physiological field affords us, through the study of wave-length and energy data, a special quantitative means of entry to a chemically inaccessible quarter. We can measure the proportion of incident light absorbed by the chlorophyll contained in the plant cells (cf. Warburg

[1] Many investigations have of course also been reported on the product of CO_2 reduction; while there are in addition numerous important methods of quite different character: morphological (cf. Frey-Wyssling, 1937,) chemical and spectroscopic investigations of plants, experiments on fluorescence (cf. e.g. Vermeulen *et al.* (1937) and Wassink *et al.* (1938), on sensitized reactions (Gaffron, 1933) and on redox systems employing chlorophyll or chemical model substances, experiments with isolated chloroplasts (Hill, 1939) and so on. A comparison of the assimilation of green plants with that of bacteria also has been of greatest value (cf. van Niel, 1931; French, 1937; Nakamura, 1937; Eymers & Wassink, 1938; Gaffron, 1935*a*). Particulars about all these lines of research may also be found in the review by Manning (1938) and Emerson (1937), and in the books of Spoehr (1926) and Stiles (1925).

& Negelein (1923), Manning *et al.* (1938), Vermeulen *et al.* (1937), Wassink *et al.* (1938), Rieke (1939), and many others partially quoted in Manning (1938), Spoehr (1926) and Stiles (1925)) which is therefore nominally available to do assimilatory work. Further, we can measure this optical internal factor, the chlorophyll content of the plant cells, in absolute units (Willstätter & Stoll (1918) and others) and can vary it in a measurable manner (cf. e.g. Emerson, 1929, 1936; Emerson & Arnold, 1932; van Hille, 1938; etc.). This is a great step forward. The other internal factors are unknown, as we have mentioned, but skilled experimenters have succeeded in controlling them so far that constant assimilation rates for a fairly long time and a reproducible dependence of assimilation on external factors could be obtained. Thus the preliminary conditions for theoretical analysis are provided.

A kinetic model is both poorer and richer than a purely chemical model. It needs chemical support, and it gives direction to chemical work just as does the thermodynamic approach, although of course the latter method is less specialized even if more sure.

A. The four-quanta process

(i) *The quantum efficiency*

Let us first assume as usual that formaldehyde is the primary product of photosynthesis. Taking as a basis the heat of reaction, the conversion of carbon dioxide into oxygen and formaldehyde requires a radiant energy of 130 kcal. mol. Now, photosynthesis is produced by all visible light up to the region of 6700 A. This means that for one photo-act there is available a quantum energy of $Nh\nu = 42.6$ kcal. mol.[1] The reduction of carbon dioxide would therefore need at least three primary photo-acts. It is interesting to know how in such a case requiring several quanta the velocity v of reaction increases with the increase of the radiant energy I. Warburg (1919) and many other authors have arrived at curves of the form of Fig. 1, curve 1. This curve starts rectilinearly. This is possible only if the energy of a quantum absorbed by chlorophyll and conveyed to the carbon dioxide molecule, is stored there until the next quantum arrives (James, 1934; see also Wohl, 1937*a*). If the first quantum could only be made use of in those cases in which the second quantum arrives during a given interval after the first, and if in all other cases its energy were in any way lost, then the curve would be S-shaped (Fig. 1, curve 2) as has been found by French (1937) for purple bacteria. We thus see that in the case of green plants the life periods of the photochemically produced intermediates are long and that there is optimum utilization of light quanta when once they are conveyed to the carbon dioxide. (Only the degree of utilization of the *last* quantum which is required for the reduction of carbon dioxide cannot be seen from the shape of the curve.) Now the light energy necessary to produce the process is in reality certain to be much bigger than the heat of reaction even if no photo-product is lost. For the return to the initial state from the activated one by emission of fluorescent light, or by dissipation in the form of heat energy may only be inhibited by additional amounts of energy (Gaffron & Wohl, 1936; Franck & Herzfeld, 1937*a*; Wohl, 1937*b*).

[1] N = Avogadro number, h = Planck's constant, ν = frequency of the light.

Thus four quanta of red light—not three quanta, as Eymers & Wassink (1938) believe—are the theoretical minimum by which photosynthesis may be achieved (Wohl, 1937*c*, *d*).

Now using small intensities of light, Warburg & Negelein (1923) found in fact that about four light quanta are needed, in the case of *Chlorella pyrenoidosa* at 10° C., to set free one molecule of oxygen. This they found, using blue light as well as red light, though in the blue region three quanta would suffice from the energetical point of view. It follows that the process of photosynthesis in red and blue light is effected by the same mechanism. (Recent work on quantum efficiency (Manning *et al.* 1938*a*,*b*; Rieke, 1939) will be reported below (p. 48), because discussion requires a number of arguments brought forward in the following pages.)

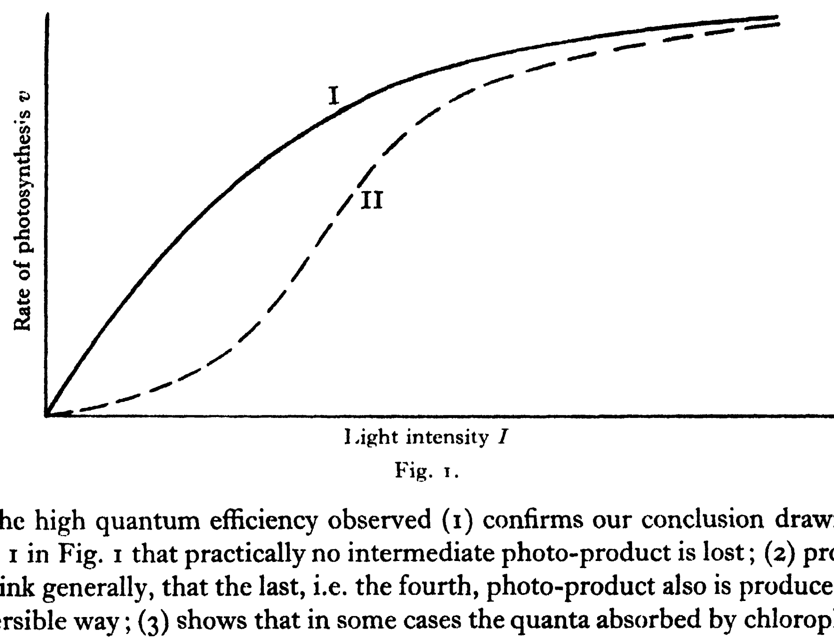

Fig. 1.

The high quantum efficiency observed (1) confirms our conclusion drawn from curve 1 in Fig. 1 that practically no intermediate photo-product is lost; (2) proves, as we think generally, that the last, i.e. the fourth, photo-product also is produced in an irreversible way; (3) shows that in some cases the quanta absorbed by chlorophyll are almost completely conveyed to the molecules of carbon dioxide capable of assimilation.

(ii) *Principal features of the mechanism*

How now does the carbon dioxide molecule store up its four quanta (Gaffron, 1933; James, 1934, 1936)? In the abstract it is equally conceivable that a carbon dioxide molecule comes to the quanta or that the quanta come to the carbon dioxide. The first possibility would mean that carbon dioxide in some form would move freely from one molecule of chlorophyll to the next, and meeting an activated chlorophyll molecule would take over its energy of activation. This mechanism of sensitization is impossible because an activated chlorophyll molecule loses its quantum energy in less than 10^{-8} sec., the smaller part being reradiated as fluorescent light, the greater part being dissipated in the form of heat energy (Prins, 1934; Franck & Herzfeld, 1937*a*). In this short interval no carbon dioxide molecule will generally collide with a chlorophyll molecule however high the concentration of

carbon dioxide in the surrounding medium may be. So we imagine that the carbon dioxide molecule, in order to be assimilated, must be firmly attached to a "reducing centre" which is coupled, in an energetic respect, to the source of quanta, that is, to the chlorophyll. It is not necessary for the purpose of the following discussion to decide whether or not the reducing centre is identical with a chlorophyll molecule.

From morphological considerations (cf. e.g. Frey-Wyssling, 1937, and Menke & Koydl, 1939) it is very probable that the chlorophyll is situated at least partially on a surface, and from the effect of surface active substances (e.g. phenylurethane) on photosynthesis (cf. e.g. Warburg, 1920) the same probably holds for the reducing centres; it is therefore assumed that the former surface contains a definite number of reducing centres which in the case of a high carbon dioxide concentration in the medium are occupied by carbon dioxide capable of being assimilated. There is still another possibility (cf. pp. 45–7) which, however, may be left aside for the time being.

Now the reaction scheme of photosynthesis can be written in the following way:

$$X_0 \xrightarrow{h\nu} X_1 \xrightarrow{h\nu} X_2 \xrightarrow{h\nu} X_3 \xrightarrow{h\nu} X_4 \xrightarrow[+H_2O+CO_2]{-O_2-CH_2O} X_0.$$

The carbon dioxide attached to the reducing centre, X_0, is transformed by four quanta taken from the chlorophyll into three intermediate products, X_1, X_2, X_3, and finally into the photo-product X_4. The latter is not sensitive to light quanta, and by a normal chemical reaction changes into oxygen and formaldehyde, giving room for the attachment of another molecule of carbon dioxide to the reducing centre. In X_4 are comprised also all states between the final photo-product and X_0 which are not sensitive to light.[1] Now the chemical reaction following the formation of X_4 has a finite velocity with a mean period of reaction τ. This we shall now compare with the mean interval t between the arrival of two light quanta at the reducing centre.

If t is much greater than τ, i.e. if the light intensity is small, then this intensity determines the velocity of photosynthesis, and we find ourselves in the first rectilinear part of the curve 1 of Fig. 1. If now carbon dioxide is present in excess approximately equal numbers of reducing centres will be occupied with each of X_0 and the three photo-intermediates, and only a small proportion occupied with X_4. For the product X_4 quickly disappears by spontaneous chemical reaction, while there is an equal probability that X_0, X_1, X_2 and X_3 will be transformed to the next photo-product by a quantum of light. If, on the other hand, the quanta follow each other in dense succession, i.e. if t is much smaller than τ, the chemical process is the slowest, thus determining the velocity of the reaction. In this case the primary product X_0 is quickly (in $4t$ sec.) transformed by the absorbed quanta

[1] In our reaction scheme arrows point only in one direction. We regard indeed the photo-reactions as essentially irreversible, as do also Burk & Lineweaver (1935). Schemes with reversible light reactions are suggested by Briggs (1933, 1935) and James (1934, 1936). From our point of view, these mechanisms deal with the reversibility only of the last (i.e. the fourth) photo act. Space does not permit of the discussion of further details in this connexion. The chemical reactions situated between X_4 and X_0, however, may be in part reversible, especially if we take into consideration the attachment of carbon dioxide to the reducing centre.

through the intermediates X_1, X_2 and X_3 into the final photo-product X_4. This, however, changes comparatively slowly (in τ sec.) into X_0, which is immediately transformed again by four quanta in X_4. So nearly all reducing centres are occupied by X_4 (including the following chemical products). Thus at each reducing centre one oxygen molecule will be formed every τ sec., or $1/\tau$ oxygen molecules will be formed every second, the rate in this case being determined by chemical processes only and independent of any further increase of light intensity. This is experimentally shown by Fig. 1, curve 1. This conception is further confirmed by the way in which photosynthesis depends on temperature at high and small intensities respectively: when the intensity is small, that is, when the velocity of reaction is determined by the intervals between the arrival of quanta, photosynthesis does not vary with the temperature. But when illumination is intense, photosynthesis shows an increase of velocity with increasing temperature, a feature characteristic of chemical reactions. This was first discovered by Blackman, and so the sum of all purely chemical or dark processes involved in the total process of photosynthesis is called the "Blackman reaction".[1]

B. THE PHOTOSYNTHETIC UNIT

(i) *The functional photosynthetic unit and the induction period at low intensities*

Concerning the connexion between carbon dioxide and chlorophyll the conception suggests itself that one molecule of carbon dioxide is coupled chemically and in an energetic respect with a single molecule of chlorophyll, and that this molecule absorbs one after the other the four quanta necessary for the reduction of the carbon dioxide molecule. This is the view of Briggs (1935) and Franck & Herzfeld (1937*b*). Let us now test this conception by means of Warburg & Negelein's experiments (1923) already mentioned on the measurements of quanta requirements.

The light intensity was so small that on an average one molecule of chlorophyll received a quantum only every 13 min. Thus one chlorophyll molecule could on an average form one oxygen molecule every $4 \times 13 = 52$ min. To ensure an optimum utilization of quanta the mean life period of each photochemically produced intermediate would require to be much longer than 13 min., say an hour (Gaffron & Wohl, 1936*a*). This seems unlikely. Quite apart from this view, however, the stationary state in the case under consideration should be preceded by a long induction period allowing of convenient observation. For if at the beginning of exposure all reducing centres are occupied by a fresh carbon dioxide molecule—and this state can be realized by suitable conditioning (Wohl, 1937*b*)—it would on an average take 52 min. before one oxygen molecule could appear. The interval in which the rate of oxygen production rises from nought to its stationary value would be still longer. Such an induction period has, however, never been observed. The period of induction described in the literature is of quite another kind. In the

[1] The effects of different chemical reagents also prove that photosynthesis consists of two different processes, light reactions and purely chemical dark or Blackman reactions (cf. e.g. Warburg 1928).

case of *Chlorella* as well as a number of other species it has a length of only 2–3 min. (cf. the review by Manning (1938)), and in general it appears chiefly at high light intensities, while our induction period according to its nature is proportional to the interval between the arrivals of successive light quanta, i.e. inversely proportional to the light intensity.

Franck & Herzfeld (1937*b*) tried to obviate this difficulty, but objections can be raised to their view (Wohl, 1937*b*). There would appear to be no other way of accounting for this lack of an induction period, at the low light intensities used by Warburg & Negelein, than by supposing that quanta meet a carbon dioxide molecule at intervals much shorter than 13 min. And this is possible in only one way: viz. if a molecule of carbon dioxide is connected in an energetic respect to quite a number of chlorophyll molecules so that no matter by which they are absorbed all quanta are transferred to one and the same carbon dioxide molecule. These considerations led Gaffron and the writer to postulate a functional photosynthetic unit, Emerson & Arnold (1932) having previously established the existence of a numerical photosynthetic unit.

(ii) *The numerical photosynthetic unit of Emerson & Arnold*

Emerson & Arnold (1931, 1932) deduced the existence of the photosynthetic unit in the following way: they irradiated green algae with short flashes of about 10^{-5} sec. duration at varying intervals. When they increased the intervals between flashes they found that the yield of assimilation per flash increased up to a constant value similar to Fig. 1, curve 1. Their explanation was that the chemical reactions following the action of light need a certain amount of time for their completion. If during this time the second flash occurs, it finds only part of the reducing centres ready to receive it. Only when the chemical reaction is completed at practically all the reducing centres at which the photo-product X_4 has been formed can a flash produce its full effect. That interval between flashes which yields an assimilation per flash e (e = the base of natural logarithms) times smaller than the maximum value, provides an approximate measure of the Blackman period. Emerson & Arnold thus found in the case of *Chlorella pyrenoidosa* at 25° C. a value below 0·02 sec. Pratt & Trelease (1938) have since made similar experiments in which the duration of flashes was longer than in the experiments of Emerson & Arnold; the dark time, however, was taken to a shorter value. They found in the case of *C. vulgaris* a Blackman period of 0·01 sec. at 24° C. which corresponds very well to the estimate of Emerson & Arnold for *C. pyrenoidosa* mentioned already.[1]

Emerson & Arnold next increased the intensity of light, while keeping the interval between flashes sufficiently long, until increase had no further effect. According to our hypothesis, each reducing centre, when light saturation has been

[1] The dependence of assimilation on the dark period which thus seems very similar for *C. pyrenoidosa* and *vulgaris* at 24° C., seems, according to measurements of Emerson & Arnold (1931), very similar for both species at 6·5° C. also. This is very striking, for the temperature coefficients of the Blackman periods for the two species are, according to Emerson & Green (1934, 1937), very different. The situation could be clarified by experiments with flashing light of very high intensity.

reached, acquires during one flash at least four quanta, so that at the end of a very short flash, each reducing centre is occupied by X_4, which afterwards is chemically transformed into oxygen and formaldehyde. (It should be pointed out that to ensure the capture of at least four quanta by almost every reducing centre it is necessary that the average number of quanta reaching each centre be much greater than four). It follows that in the case of saturation one carbon dioxide molecule per reducing centre per flash is reduced. Thus can be written $\Delta_{max.} = n$, where $\Delta_{max.}$ is the maximum yield of assimilation per flash and n the total number of reducing centres present. If now one reducing centre were identical with one chlorophyll molecule, then $\Delta_{max.} = \nu$ should be valid, ν being the total number of chlorophyll molecules present. For a great number of plants Emerson & Arnold (1932) and Arnold & Kohn (1934) always found

$$\Delta_{max.} = n = \frac{\nu}{2500}. \tag{2}$$

This means that in these plants for each reducing centre there are about

$$\frac{\nu}{n} = Z = 2500 \tag{3}$$

chlorophyll molecules. This is the number we call the numerical photosynthetic unit.

(iii) *The functional photosynthetic unit and the assimilation/light intensity curve*

The size of the numerical photosynthetic unit Z in itself does not reveal how many of these 2500 chlorophyll molecules co-operate in photosynthesis. At first it was assumed that this number was very small; that would mean that the magnitude of the functional photosynthetic unit is close to unity, and that the efficiency of chlorophyll being obviously z/Z, is very small.

Quite apart from the inconsistency of this view with Warburg & Negelein's observations on very small light intensities, the value of the functional photosynthetic unit z may be ascertained numerically from the variation of the rate of assimilation with the intensity of light. In continuous illumination this variation is given by Fig. 1, curve 1. The situation at small intensities has already been amply discussed. It has also been mentioned that at very high intensities on an average one oxygen molecule is formed at the reducing centre during one Blackman period τ. Thus $1/\tau$ oxygen molecules have developed per second per reducing centre, i.e. $v_{max.} = n/\tau$ oxygen molecules per second overall. Dividing this measurable quantity by ν, the equally measurable total number of chlorophyll molecules

$$\frac{v_{max.}}{\nu} = \frac{n}{\tau\nu} = \frac{1}{\tau Z}. \tag{4}$$

results. This is the maximum number of oxygen molecules formed per chlorophyll molecule per second. Willstätter & Stoll (1918) and Emerson & Arnold (1932) found this number to be about 1/30 sec.$^{-1}$ at 25° C. The reciprocal of this value, $\tau Z = 30$ sec., was called "time of assimilation" by Willstätter & Stoll.

In the alga *C. pyrenoidosa*, Emerson & Arnold (1932) combined the equation $\tau Z = 30$ sec. with the equation $Z = 2500$ given above, thus arriving at a fairly exact value for the Blackman period at 25° C., viz. $\tau Z/Z = \tau = 30/2500 = 0{\cdot}012$ sec.

Transferring this value of the Blackman period to the plants with which Willstätter & Stoll observed the time of assimilation τZ, similarly in these cases definite values for the numerical photosynthetic unit Z are arrived at. The result is given in lines 1 and 4 of Table 1, which has been taken, with some alterations (Gaffron & Wohl, 1936*b*; Wohl, 1937*a*), from the paper by Gaffron and the writer (1936*a*). The data are taken from Willstätter & Stoll (1918) for the leaves, from Emerson (1929*a*, *b*) for *C. vulgaris* and from Emerson & Arnold (1932) and Warburg & Negelein (1923) for *C. pyrenoidosa*. Table 1 shows that except for the yellow varieties of elm and elder the numerical photosynthetic unit is always of the same order of magnitude.

Table 1[1]

		Ulmus, green	*Sambucus nigra*, green	*Ampelopsis*, autumnal	*Ulmus*, yellow	*Sambucus nigra*, yellow	*Chlorella vulgaris* High concentration of chlorophyll	*Chlorella vulgaris* Low concentration of chlorophyll	*Chlorella pyrenoidosa*
1	$Z\tau$ sec.	17·9	24·9	71	1·96	1·18	35·2	28·3	30
2	$z\tau$ sec.	13	31	14	2·3	2·1	3·0	2·3	(30)
3	z/Z	0·73	1·25	0·197	1·17	1·78	0·085	0·081	(1)
4	Z	1490	2080	5900	163	98	2930	2360	2500
5	z	1080	2580	1160	192	175	250	192	(2500)

[1] Cf. list of symbols at the head of this paper.

We may now consider the state in which the velocity is half the maximum velocity, i.e. in which half an oxygen molecule is formed per reducing centre per Blackman period. In this state one reducing centre must be met on an average by four quanta during the time equal to the length of the Blackman period. The reason is that this time is followed by the Blackman period itself during which four quanta also arrive but remain without effect. In this way one oxygen molecule is formed in 2τ sec. by eight quanta, or half an oxygen molecule in τ sec. by four quanta.

So we can say that in the case of an intensity producing half-maximum rate of assimilation the number of light quanta reaching one reducing centre per τ sec., or the number of effective quanta per reducing centre per second, equals 4. If the number of effective quanta per second at half-maximum rate be written $N_{(\frac{1}{2})\,\mathrm{eff.}}$ this may be expressed

$$N_{(\frac{1}{2})\,\mathrm{eff.}}\,\frac{\tau}{n} = 4. \tag{5}$$

Now the number of effective quanta equals the product of the number of all quanta multiplied by the efficiency of chlorophyll: $N_{(\frac{1}{2})\,\mathrm{eff.}} = N_{(\frac{1}{2})}\,.\,z/Z$. On the other hand, the number of reducing centres is $n = \nu/Z$ (equation (3)). Introducing both expressions in the equation (5) we get

$$\frac{N_{(\frac{1}{2})}\,.\,z/Z\,.\,\tau}{\nu/Z} = \frac{N_{(\frac{1}{2})}\,.\,z\tau}{\nu} = 4 \quad \text{or} \quad \tau z = \frac{4\nu}{N_{(\frac{1}{2})}}. \tag{6}$$

The results for τz are given in the second line of Table 1. These can be utilized in two ways. Dividing τz by τZ, the efficiency z/Z of chlorophyll is obtained which proves to be of the order of unity (the theoretical maximum) except in case of *C. vulgaris* where it is equal to 1/12. (The relatively low photo-activity of *C. vulgaris* has been pointed out by Emerson (1936) and Emerson & Green (1937) (see also Gaffron & Wohl, 1936*b*)). For *C. pyrenoidosa* the value unity has been taken in view of the quantum efficiency determined by Warburg & Negelein.[1]

From the values of Z in the fourth line of Table 1, and from z/Z, the functional photosynthetic unit z as given in the fifth line may be derived. There is still another approach to z: it is known from Emerson & Arnold's experiments employing short intervals between flashes (1931) that in the case of *C. pyrenoidosa* the Blackman period τ is less than 0·02 sec. Transferring the result of Pratt & Trelease (1938) obtained for *C. vulgaris* to the related alga a value of 0·012 may be roughly estimated. Introducing this value in the equation $\tau z \cong 30$ the result $z \cong 2500$ is obtained. Using the data available, this observation is less exact than the previous one, but it is important because it does not depend in any way upon the numerical photosynthetic unit Z.

(iv) *Physiological aspects*

Considering the biological utility of the photosynthetic unit, we must start from the point of view that the number of reducing centres in the chloroplast has a biologically fixed limit which may be connected with the later fate of the products of assimilation. If this be the case the collecting of light quanta by the functional photosynthetic unit is obviously of great biological value. The photosynthetic unit is generally so proportioned that the rate of assimilation in diffused daylight is not far from the maximum rate.

Table 1 suggests a simple explanation of the difference between assimilation of normal yellow and autumnal leaves as well as of that between sun and shade plants (Gaffron & Wohl, 1936*a*). In autumnal leaves some of the reducing centres have lost their fitness for photosynthesis. Therefore the number Z of chlorophyll molecules present per *active* reducing centre has been increased. The functional unit z, however, has not altered. In yellow leaves the number of reducing centres has not been decreased in the same degree as the number of chlorophyll molecules, the latter forming small but highly efficient units around the reducing centres.

So autumnal leaves can furnish the reducing centres with sufficient light when illumination is weak but are not efficient in strong light. On the other hand, yellow leaves are not efficient in weak light but can utilize their content of chlorophyll very well in intense light.

It is doubtful if a similar difference exists between sun and shade plants, i.e. if shade plants are characterized by few reducing centres and big units, sun plants

[1] Incidentally, it may be mentioned that we might have calculated z from the rectilinear part of Fig. 1, curve 1 at low intensities and from the maximum rate of assimilation, using the equation

$$\tau z = \frac{v}{v_{max}} \cdot \frac{4\nu}{N}, \tag{7}$$

N being of the number of quanta per second. Equation (7) is an analogue of equation (10).

by many reducing centres and small units. The interesting observation of Emerson (1936) that an enlargement of the cells causes an increase of assimilation rate though the amount of chlorophyll per cell was not changed, means from our point of view that during the period of the investigation, while the amount of chlorophyll remained constant, the number of effective reducing centres increased.

It might seem possible to explain most of these differences with the aid of the Blackman period, i.e. by postulating different concentrations of enzymes or other chemical variations. But as *C. pyrenoidosa* and *C. vulgaris*, in spite of their very different photosynthetic efficiency, seem to have the same Blackman period (Emerson & Arnold, 1931; Pratt & Trelease, 1938), the explanation given above seems more plausible. The method of Kohn (1936), described immediately below, could be used as an *experimentum crucis*, since it yields direct information on the functional photosynthetic unit z instead of the product τz. Finally, the observation on the connexion between photosynthesis and chlorophyll content of the cells recently published by van Hille (1938) can probably be better explained by a variation of the size of the photosynthetic unit than by the alteration of the Blackman period suggested by the author. Another problem which might possibly be solved using the concept of the photosynthetic unit is raised by the same author in the words: "There must exist—in spite of the independence of chlorophyll content and Blackman reaction—an internal factor in the green plants which prevents the assimilation numbers to diverge widely, a fact which is not yet understood."

(v) *The functional photosynthetic unit and flashing light*

A method similar to that described above for evaluating the functional photosynthetic unit has been applied by Kohn (1936) to experiments of Kohn & Arnold (similar to those published by Arnold & Kohn, 1934), in which assimilation in flashing light with long intervals has been determined in relation to the intensity of the flashes. Here the Blackman period was not a disturbing factor, and assimilation per reducing centre is solely a function of the number of quanta taken up per flash by the reducing centres.

At the highest intensity of the flash $\Delta_{\text{max.}}$, according to equation (2), equals n, i.e. the number of oxygen molecules formed is equal to the number of reducing centres. At very small intensities of the flash on the other hand, each reducing centre receives at most one quantum per flash. As the intermediate photo-products endure a sufficient length of time, no quantum is lost, and at the beginning of one flash the reducing centres are equally shared among the products X_0, X_1, X_2, X_3, just as in the case of weak continuous illumination. Only a quantum which hits the reducing centre occupied with X_3 can produce X_4 and consequently oxygen, and make possible the attachment of another carbon dioxide molecule. Thus for the small intensities just spoken of the number of oxygen molecules produced by one flash equals $\frac{1}{4}$ of the number of the effective quanta per flash $\mathbf{N}_{\text{eff.}}$. It follows

$$\Delta = \frac{\mathbf{N}_{\text{eff.}}}{4}, \tag{8}$$

and furthermore (cf. equation (2))

$$\frac{\Delta}{\Delta_{\text{max.}}} = \frac{N_{\text{eff.}}}{4n}. \tag{9}$$

According to the equations following equation (5) it may be written

$$\frac{\Delta}{\Delta_{\text{max.}}} = \frac{N.z/Z}{4\nu/Z} = \frac{Nz}{4\nu},$$

and finally

$$z = \frac{\Delta}{\Delta_{\text{max.}}} \cdot \frac{4\nu}{N}. \tag{10}$$

In this way $z \cong 2500$ can be derived in agreement with the calculation in § B (iii). This calculation, however, would only be exact if Kohn had made chief use of the slope of the curve at lower intensities of the flashes. Otherwise, z is reduced by a definite though hardly considerable amount. Kohn himself (1936), for the sake of certainty, used the maximum value of the absorption coefficient of chlorophyll instead of a medium value and omitted the factor 4 on account of an incomplete theory, thus getting the equation $z = 360$.

Summing up, we may say that all experiments by which the theory of the photosynthetic unit has up to now been checked have spoken in its favour.

(vi) *Other theories*

Briggs (1933, 1935) has discussed a highly interesting and useful scheme of assimilation with which he explained the experiments of Emerson & Arnold (1931, 1932) without making use of Emerson & Arnold's conception of the *numerical* photosynthetic unit. I believe, however, that Briggs's explanation does not fit the facts which have led to the establishment of a *functional* photosynthetic unit. Apart from certain difficulties arising at higher intensities there should be observed according to Briggs's scheme a long induction period under the conditions of Warburg & Negelein's experiment, but, as has been mentioned, no such period was found (Wohl, 1937*b*). Therefore the scheme will be valid only in principle but not quantitatively, and the theory of Briggs does not make the photosynthetic unit superfluous.

More recently, Franck & Herzfeld (1937*b*) have given an explanation of the facts mentioned above which differ from the present explanation. They did not do so because of any objections to the theory of the photosynthetic unit developed above, but because they did not think it possible that there could be a physically plausible mechanism of the photosynthetic unit.

In order to understand the theory of Franck & Herzfeld we must remember that the photosynthetic unit works at high intensities of light in such a way that the assimilation/light intensity curve becomes horizontal much earlier, and that the maximum assimilation rate is much lower than we should expect if there were no such unit. Both facts are explained by Franck & Herzfeld in the following way: a photochemical product which normally undergoes a chemical transformation releasing oxygen will, during its life period, frequently be hit by another quantum

if the light intensity is sufficiently high, and will disintegrate, setting free a radical. This radical is supposed to move freely on a surface covered with chlorophyll molecules, there to meet other similar photo-products and to destroy these catalytically in a chain reaction. This is supposed to go on happening until the radical itself somehow disappears. Thus the rate of assimilation would be diminished at high intensities according to the length of the reaction chain.

Franck & Herzfeld showed that to explain the experimental data it has to be assumed that at high intensities of light one radical during its life period destroys about 500 photo-products, and they showed further that the assimilation curve may become horizontal at high intensities only if the radicals disappear by a *monomolecular* reaction. But a calculation shows that, at concentrations which must be assumed in order to explain the experimental results, the radicals disappear by mutual bimolecular reaction at such a rate that at fairly high intensities a radical gets on an average no opportunity at all of colliding with even one single photo-product. Thus this ingenious explanation has to be abandoned.

Besides, we have already pointed out in discussing the theory of Briggs that the absence of any induction period at small intensities of light cannot be explained at all on this basis but demands a theory where quanta are accumulated, that is to say, a theory involving the photosynthetic unit.

(vii) *The kinetic model of the photosynthetic unit*

How now can the co-operation of about 2000 molecules of chlorophyll with one carbon dioxide molecule be conceived? Of course this question cannot be answered fully, but it seems worth while to attempt some conception in order to see whether the idea of the photosynthetic unit is a physically sound one. It has already been shown that it cannot be assumed that the carbon dioxide molecule moves freely, collecting quanta, from one activated chlorophyll molecule to the next. Neither is it possible for a molecule functioning as an intermediate carrier, to collide with an activated chlorophyll molecule, to take over its quantum and to bring it to the reducing centre. But we may consider (Wohl, 1937*d*) whether the absorption of a light quantum by chlorophyll could bring about the setting free of a radical which would travel to the reducing centre and give up there its chemical energy. For this to be the case the radical, having lost its additional energy, would subsequently have to leave the reducing centre and again join a chlorophyll molecule. If we take it as an experimental result that the ratio of reducing centres to chlorophyll molecules in the cell is 1 to 2500, then one carbon dioxide molecule might obviously in this way gather up the quanta absorbed by 2500 chlorophyll molecules. But in our case the conception of real radicals seems to be rather questionable, because radicals on recombining with each other will always diminish the quantum efficiency. Moreover, the biological environment is so sensitive that we can hardly assume the radicals to exist undisturbed and to be biologically innocuous. These difficulties are diminished if we assume that the chlorophyll has taken up some substance which on absorption of a light quantum dissociates from the chlorophyll molecules in an isomeric form of high energy. These high-energy molecules must be supposed to

have sufficient life period and stability mutually against one another and against the environment to travel unchanged to the reducing centres and there discharge their energy specifically by collision. The chlorophyll after dissociation would have to join another molecule of the same kind which must be present in abundance. Whether the reducing centre lies on a surface or is a dissolved molecule is irrelevant to this conception.

In the case of continuous illumination the medium between chlorophyll and reducing centres contains activated "vehicular" molecules in a concentration depending on the intensity of light. This concentration may be approximately estimated. Even at extremely high intensities it has a reasonable value of about 1/1000 mol. per litre. It is an interesting point that this conception involves a noticeable interval between the moment the light quantum is absorbed and the moment its energy is taken over by the carbon dioxide molecule. This interval we may call the photo-period in which the vehicular molecules travel from the chlorophyll to the reducing centre. Now in experiments with continuous illumination this is of no importance, as a stationary concentration of the activated molecules is maintained, and the induction and extinction periods of this stationary concentration are of course immeasurably small.

This conception seems also to be applicable without great difficulty to the experiments in flashing light. Let us consider in the first instance experiments with a long interval between very short flashes. Here the main effect will start when the flash has already vanished, i.e. when the vehicular molecules have travelled on an average the distance from the chlorophyll molecules to the reducing centres. If these flashes are exceedingly intense a definite concentration of vehicular molecules will be brought about which will afterwards fade away in about the time of the photo-period. In the experiments in question this will likewise be unnoticeable.

In cases, however, where the interval between flashes is shortened the degree to which assimilation is diminished will vary according as the photo-period ϑ is or is not short compared with the Blackman period τ. In these experiments the existence of the photo-period is without importance only if ϑ is shorter than τ. We know the Blackman period at 25° C., to be 0·012 sec., and to increase considerably as the temperature is lowered. Let us now imagine that the chlorophyll molecules are lying closely crowded on a surface (cf. Frey-Wyssling, 1937; Mommaerts, 1938; and Nicolai & Weurman, 1938) that the reducing centres are lying on the same surface, and that the vehicular molecules are moving across the surface. Then the photo-period may in fact equal about 1/30 of the Blackman period at 25° C. We get the same small value for the photo-period if we imagine two such surfaces closely crowded by chlorophyll molecules to be lying opposite each other at a distance of about 115 A.,[1] and the vehicular molecules to be moving in three dimensions through the medium between these surfaces.

There is still one further possible modification of the kinetic mechanism according to which the characteristic period of 0·012 sec. at 25° C., derived from experiments in inter-

[1] The values correspond to an area of the chlorophyll molecule of 110 Å^2 and differs from Wohl (1937*d*). Such structures have been suggested by Frey-Wyssling (1937) and Hanson (1939).

mittent light, or from a comparison of experiments in intermittent and continuous light, is not at all the true Blackman period which limits the rate of photosynthesis in continuous light. This period rather has the significance of a mean life time of vehicular molecules, the Blackman period being shorter. Then the famous period of 0·012 sec. deals with the process furnishing a carbon dioxide molecule with quanta but not with a process utilizing the quantum energy received for the work of chemical reduction. Further particulars and possibilities opened up by this proposed mechanism have been discussed in Wohl (1937*d*, *e*).

Whether one of the proposed kinetic mechanisms of the photosynthetic unit will hold good can of course not be predicted with any degree of certainty, but so far none seems to have been disproved. Emerson (1937) is inclined to assume a photosynthetic mechanism of the type of the kinetic model developed above. Ornstein *et al.* (1938) say: "It is necessary to separate chlorophyll completely from the chemical reactions. This can only be reconciled with a demand of a high efficiency if it is accepted that the chlorophyll transmits its energy to a compound present in abundance. A conception fulfilling this requirement is a modification of the schema of Gaffron & Wohl recently proposed by Wohl as kinetic model of the photosynthetic unit." The authors have adopted and specified the modification of the kinetic model last mentioned and have applied it quantitatively to experiments with continuous light.

(viii) *The optical model of the photosynthetic unit*

Quite another possibility of a mechanism of the photosynthetic unit seems to exist. This has been proposed by Gaffron & Wohl (1936*a*), and has been challenged by Franck & Herzfeld (1937*b*) and recently by Manning (1938), but has in the meantime, at least in principle, been accepted in different quarters. This is the so-called optical mechanism of the photosynthetic unit. Here about 2500 molecules of chlorophyll are supposed to form a solid structural unit, having at one place a reducing centre for carbon dioxide—this may be one of the 2500 chlorophyll molecules, or as Gaffron (1933, 1937) thinks, a molecular group adjacent to the chlorophyll molecule. A light quantum which has been absorbed by one of the chlorophyll molecules is supposed to fluctuate practically without loss through the body of the photosynthetic unit, in such a way that its energy may be captured by the reducing centre where the carbon dioxide molecule is situated. Later on, Delbrück (personal information) found that a body with such qualities is physically conceivable. If the intervals between the absorbing centres of the chlorophyll molecules of the body are of atomic dimensions, or in other words, if the chlorophyll molecules are packed flatly one over the other in such a manner that all absorbing centres are in direct contact, then this crystalloid structure will be activated as a whole as soon as a light quantum is absorbed at any point.

This idea of the optical photosynthetic unit should hold good also for crystals where it may be assumed that there is no limitation of the optical unit to the number 2500 (Wohl, 1937*d*). The same theoretical point of view is shared by Scheibe (1937), who assumes that polymeric products of dyestuff molecules behave in many ways like the optical photosynthetic unit, and that this behaviour

accounts for the effect of sensitizers of photographic plates to the infra-red. Riehl (1939) accordingly supposes that the activation of the phosphorescent substances of sulphide of zinc is transmitted undisturbed over twenty layers of the lattice to the reradiating centre. Möglich & Schön (1938) are of the same opinion. Timoféeff & Delbrück (1936) have found from experiments on the effect of X-rays on chromosomes that a mutation is caused if from 1 in 100 to 1 in 1600 of the atoms which make up the chromosome is activated, mutation consisting of a single elementary act, as has been shown by Timoféeff *et al.* (1935). Lately, Timoféeff and Zimmer (personal information) have come to the conviction that in the genes certain structural elements are reiterated, and the genes may therefore be taken to possess some kind of crystalloid structure. The authors are therefore inclined to explain the abnormal extension of the area of sensitivity by a direct transmission of electronic energy over many molecular intervals, just as in the case of the optical photosynthetic unit. Jordan (1938*a*) also assents to our view and looks upon the optical photosynthetic unit as an example of the importance of what he calls a "micellar" structure.

(ix) *Recent work on quantum efficiency in photosynthesis*

The result of Warburg & Negelein (1923) on quantum efficiency in the case of *C. pyrenoidosa* (cf. p. 36) has recently been confirmed by Rieke (1939) in an excellent manner. Rieke showed that 4·2 quanta of yellow light are required for the reduction of one carbon dioxide molecule. On the other hand, Manning *et al.* (1938) investigating the same alga, found that 16–20 quanta are needed. Apart from the different experimental methods, the main departures from the conditions in the experiments of Warburg & Negelein and Rieke are as follows: (1) Considerably longer light and dark times, and a long pause between the measurements of photosynthesis and respiration; (2) the use of an intensity of light and a concentration of algae giving a rate of assimilation stronger than that of respiration, while in the former experiments respiration prevailed; (3) a temperature of 25° C. instead of 10° C.

Manning *et al.* (1938) discussed whether the intermediates of the photo-process, at the high temperature and low-light intensity of their experiments, might break down partially before they were met by another quantum and were thus utilized in the photosynthetic process. They did so on account of the existence of an induction period which would be caused by the necessity for the reformation of intermediates broken down in a dark period. The kind of induction period to which the authors refer only exists at high light intensities, as is shown by Warburg (1920) and McAlister (1937). According to Smith (1937), it is true, the induction period does not vanish when the intensity is diminished, but Manning (1938) has pointed out that the smallest intensity used by Smith was still far higher than that at present in question. Emerson (1936) states that his experiments "do not indicate the presence of an induction period which becomes shorter at higher intensities." At least this statement is beyond doubt (cf. p. 39).

Now if the induction period were occupied with the reformation of the photo-intermediates its length should be proportionate to the interval between the arrival of successive quanta building up these intermediates, and therefore it should be immeasurably small at high intensities at which it has been observed, and should increase as the intensity decreases which has not been observed. Indeed, there can be no connexion between the duration of the induction period at high intensities and the quantum intervals at low intensities. Actually the induction period necessary to build up the photo-intermediates is of quite another character than that just mentioned, and it must exist even if the intermediates are

absolutely stable. The problem whether it is observable at small light intensities, and the importance of this problem with respect to the photosynthetic unit, have been discussed by Gaffron & Wohl (1936*a*) and by the writer (1937*b*).

The explanation of the divergent values obtained for the quantum efficiency on the basis of the decay of photo-intermediates is opposed, as Manning *et al.* (1938*a*, *b*) point out, by the observation of Warburg (1919) that photosynthesis is independent of the temperature at light intensities only slightly higher than those used in the quantum efficiency experiments. (Incidentally the kinetic model of the photosynthetic unit (Wohl, 1937*d*) shows that the quantum efficiency at low-light intensities might depend on temperature without depending on the interval between the arrival of successive quanta. It may be added that the loosening of the structure of the optical model of the photosynthetic unit at increasing temperature would give the same effect.) Moreover, Manning *et al.* (1938*a*), in an experiment of the same kind as those of Manning *et al.* (1938*b*) though not quite so accurate, have found that the quantum efficiency at 10·8° C. (practically the temperature of Warburg & Negelein's experiment (1923)) is as low as was found (Manning *et al.* 1938*b*) at 25° C. Now Warburg & Negelein used a very dense suspension of algae which was vigorously shaken, while Manning *et al.* used a relatively thin layer of algae. In this case equal intensity of incident light may bring about quite unequal light conditions. For a single chlorophyll molecule, at the intensity used by all investigators mentioned, absorbs a light quantum according to Manning *et al.* once every 30 sec. Now if we assume that one carbon dioxide molecule obtains its quanta only from one chlorophyll molecule to which it is attached, then one carbon dioxide molecule which has obtained one quantum in Warburg & Negelein's experiment, will have been washed away from the absorbing zone in front of the vessel long before the next quantum arrives. Consequently the whole of the suspension can be regarded as equally illuminated, so that one carbon dioxide molecule will receive one quantum every 13 min., as shown by Gaffron & Wohl (1936*a*), (cf. this paper p. 38),[1] while the interval in the experiments of Manning *et al.* might be 1 min. Thus a short life period of intermediates ought to result in a low quantum efficiency in Warburg & Negelein's experiment compared with that of Manning *et al.*, whereas actually a far higher efficiency was found. Assuming the existence of a photosynthetic unit; one carbon dioxide molecule under the same conditions obtains its light quanta about once every 0·02 sec., so that illumination in Warburg & Negelein's experiment becomes irregular and our argument consequently becomes weak. (If it could be established independently, e.g. by flashing light experiments, that the life period of photo-intermediates is of the order of magnitude of seconds or minutes (Emerson & Arnold, 1931, 1932; Wohl, 1937*a*), this argument would speak in favour of the existence of the photosynthetic unit.) In any case, as Manning *et al.* (1938*b*) have pointed out, down to the illumination used in their own experiments, no decrease of quantum efficiency caused by the decrease of light intensity has been observed.

It is also improbable that the discrepancy could be accounted for by the fact that the experiments of Manning *et al.* (1938*b*) were carried out above the point at which assimilation and respiration compensate one another, while those of Warburg & Negelein and Rieke were carried out below that point. For Warburg (1920) has made it clear that the assimilation/light intensity curve shows not a trace of break or bend at the point of compensation. Rieke (1939) also refers to this point, and Eymers & Wassink (1938) emphasize the same fact in the case of purple sulphur bacteria. Van der Paauw (1935), being of another opinion, does not take this argument into consideration. Even the relation of the point of compensation to the effect of HCN (Warburg, 1920; van der Paauw, 1935), is not a general feature of photosynthesis (van der Paauw, 1932; Gaffron, 1937), and is regarded by Gaffron (1937) as a kind of chance which he has explained in another way.

A difficult problem is presented by the question whether or not the increase of respira-

[1] Eymers & Wassink (1938) unfortunately do not elaborate their objections to this point.

tion during the time of the illumination has an influence on the experimental values of quantum efficiency. In any case light respiration cannot be significant in Warburg & Negelein's experiment (1923), for otherwise the real quantum efficiency would become higher than is possible from the energetic point of view. Warburg himself (1921) found that the quantum efficiency was diminished to half its value by altering the temperature from 5 to 20° C. He gave the following explanation: at 5° C. the rate of respiration during the short dark periods employed would be considered equal to the rate during light periods, because of the slow rate at which the value fell after the cutting off of light, whereas at 20° C. the fall was so rapid that the two rates could no longer be considered equal. Van der Paauw (1932) and Petering & Daniels (1938), the latter measuring only oxygen output, also found that light respiration could be observed at moderate light intensities during short periods. On the other hand, Emerson (1935), Gaffron (1937) and McAlister (1937), the last measuring only carbon dioxide intake,[1] did not find any light respiration at all; Eymers & Wassink (1938) also failed to observe, for purple bacteria, an increase of the essential dark metabolism by light. As Manning *et al.* (1938 *a*, *b*) either used long periods of observation or determined respiration by a parallel experiment in the dark; it is very possible that light intensity may have been a disturbing factor. It is quite unlikely, however, that this point is responsible for the enormous difference between the quantum efficiencies observed.

Recently it has been found that in the case of some species of purple bacteria also, four quanta are needed for the assimilation of one carbon dioxide molecule (Nakamura, 1937; French, 1937), though for another species (Eymers & Wassink, 1938) at least 12 quanta are needed. The observations of French are extremely interesting: he has obviously found an example in which the life period of one or more of the photo-intermediates is sufficiently short for a curve of the shape of II, Fig. 1 to be produced instead of curve I. The argument of the author against this view cannot be considered conclusive.

French succeeded in lengthening the mean life periods of the intermediates by pre-treatment, so that the curves became very similar to curve I, Fig. 1. This seems not to be unlikely, as the breakdown of the intermediates may be caused by enzymes in the medium, the state and concentration of which can depend on pre-treatment. The number of quanta needed for the reduction of carbon dioxide was as high as 8 for curves of type II, Fig. 1, and diminished to the value 4 in an apparently asymptotic manner as the curve approached type I—which would theoretically be expected.

Further it was observed that with increase of temperature the curve moved in the direction from type I to type II. This result is also to be expected, as has been pointed out by Manning (1938), since the higher conditions of temperature would be more favourable to the breakdown of intermediates.

The writer cannot follow Emerson (1937), Manning (1938) and Eymers & Wassink (1938) in their objections to French's result. On the other hand, the bearing of the observed time effects on the other curves obtained by French does not seem to be completely clarified in his paper. Yet it must be agreed that the findings of French support van Niel's (1931) view that the mechanism of photosynthesis may be essentially a four-quanta process, even in cases in which the chemical and energetic conditions are quite different from those obtaining in green plants.

To sum up: since Warburg & Negelein's work (1923) has been confirmed experimentally by Rieke (1939), and since there is no theoretical reason why these measurements should not be conclusive, it has to be taken as proved that in their experiments only about 4 quanta were used for the reduction of each molecule of carbon dioxide.

[1] The different results obtained by Petering & Daniels and by McAlister indicate that the photosynthetic quotient O_2/CO_2 of light respiration should be investigated. The same has been suggested for the induction period in Wohl (1937*b*).

That the quantum efficiency is frequently less is well known: see e.g. Burns (1938), white pine trees, and Briggs (1929) leaves. But what is important for the theory is the fact that it has been possible in any instance to demonstrate the high efficiency. For we assume that the mechanism of photosynthesis is essentially the same in all green plants, and that accidental circumstances—worth while to be investigated in their turn (Eymers & Wassink, 1938), of course—diminish its efficiency in particular cases.

C. The sequence of photo processes and dark reactions

In the first part of this paper, the following reaction schema has formed the basis of the deductions:

$$X_0 \xrightarrow{h\nu} X_1 \xrightarrow{h\nu} X_2 \xrightarrow{h\nu} X_3 \xrightarrow{h\nu} X_4 \xrightarrow[+H_2O+CO_2]{-O_2-CH_2O} X_0.$$

This schema still contains one uncertainty which, however, does not affect the main substance of our reasoning. It is conceivable that the four light reactions do not follow each other uninterruptedly, but that in between them there take place chemical reactions (cf. e.g. Burk & Lineweaver, 1935). During these chemical reactions the system would not be sensitive to light quanta. We should thus have an alternating sequence of Blackman processes and photo processes. This question is of no importance for the rate of assimilation under continuous illumination. Here only one apparent Blackman period can be observed which is the sum of all mean partial reaction periods, irrespective of their mutual sequence and their position between the photo processes.

This is, however, not the case with experiments on flashing light of high intensity (Wohl, 1937*a*). One has only to consider the experiments with long intervals between the flashes[1] (Emerson & Arnold, 1931, 1932). Imagine the intensity of the flashes to be increased gradually from very small values. It is known that at small intensities the rate of assimilation independently of any Blackman period is always equal to one-quarter of the number of quanta absorbed by the reducing centres. Now, if all photo processes were separated by Blackman periods longer than the duration of the flashes, i.e. longer than 10^{-5} sec., even at very high intensities each reducing centre would utilize only one light quantum out of each flash. All other quanta would arrive during the Blackman periods and thus be without effect. As the production of one oxygen molecule needs four quanta, four flashes at least are necessary to form one oxygen molecule at one reducing centre, i.e. the maximum rate of oxygen formation per flash is equal to one-quarter of the number of reducing centres. If, on the other hand, the system is uninterruptedly ready to take up light quanta until the fourth quantum has arrived, i.e. if no chemical reactions or only reactions with mean periods much shorter than 10^{-5} sec. occur between the flashes, then the maximum number of oxygen molecules formed per flash is equal to the number of reducing centres itself as was assumed before.

If it is found that the maximum yield of assimilation is equal to 1/2500 of the number of chlorophyll molecules, the number of reducing centres in the case of alternating photo and Blackman processes will be 1/625 of the number of chlorophyll

[1] The experiments available with shorter dark periods cannot assist in answering the present question; Wohl (1937*a*).

molecules, or Z will be equal to 625. Assuming an uninterrupted sequence of the photo reactions Z is 2500 as we know. The experiments on continuous illumination give just as before $Z=30$. So in the first case the total Blackman period τ is equal to $30/625=0{\cdot}048$ sec., in the second case equal to $30/2500=0{\cdot}012$ sec. as already shown. It should be possible to decide between these alternatives from the shape of the assimilation/intensity curve as its initial slope is the same in both, but the final value in the second is four times that in the first.

In the experiments on assimilation and flashing light carried out by Emerson & Arnold (1931, 1932) the absolute intensity of the flashes was not known. Let us

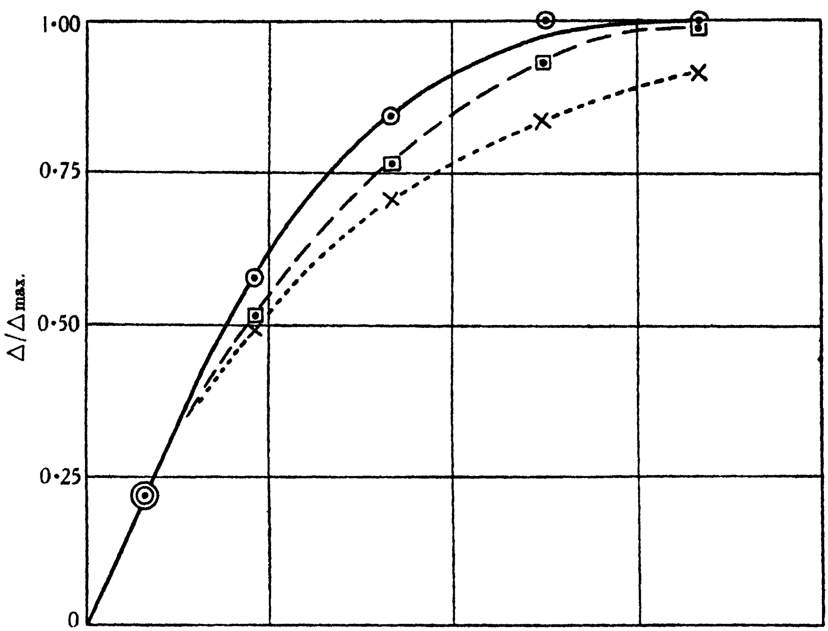

Fig. 2.

—⊙— Experimental data.
—□— Uninterrupted sequence of photo processes.
. . × . . Alternating sequence of dark and photo processes.

therefore compare both theoretical assimilation/intensity curves with the data in the following way, which is in principle the same as used before (Wohl, 1937*a*). The ordinates will be the ratio of the yield of assimilation at any point to the maximum yield, and the scale of the abscissae which represent light intensities is so adapted that all three curves have the same initial slope. Fig 2 shows how the curves differ at higher intensities. The comparison is clearly in favour of the theory of an uninterrupted sequence of the photo processes. It is true that at medium intensities the observed curve differs noticeably from *this* theoretical curve also. Emerson & Arnold (1932) assume it to be possible that at their highest intensities the maximum value of assimilation is not yet reached though, the writer feels, the shape of the

observed curve tells against such an assumption. If they were correct in their supposition their curve would agree better with the theoretical one. Further tests which support the scheme suggested on pp. 37 and 51—which also seems to be simpler from the chemical point of view—will not here be mentioned (cf. Wohl, 1937*a*).

D. THE BLACKMAN REACTION

(i) *The dependence of the maximum rate of assimilation on temperature*

The rate of photosynthesis at high intensities of continuous light is determined, as is known, by the Blackman period. It will be remembered that as the concentration of carbon dioxide increases assimilation becomes independent of it. This proves that the limiting chemical factor *in high concentrations of carbon dioxide* is to be found neither in the number of collisions between carbon dioxide molecules and the reducing centre, nor in the concentration of a compound formed by their combination, nor even in a velocity of diffusion caused by a fall in concentration of carbon dioxide.

In this section an attempt will be made to learn something of the nature of the Blackman period from the dependence of the maximum rate of assimilation on temperature at high light intensity and high carbon dioxide concentration (Wohl, 1937*c*). The maximum velocity of photosynthesis is given by $v_{\max.} = n/\tau = nv_n$ (cf. p. 40), i.e. it equals the number of reducing centres n, either divided by the Blackman period τ, or multiplied by the number v_n of reactionary events per second at one reducing centre. n being constant, the relative change of $v_{\max.}$ or the change of the logarithm of $v_{\max.}$ is identical with that of $1/\tau = v_n$. If we were here dealing with a single chemical reaction, then the maximum rate of assimilation ought to depend on temperature according to Arrhenius's equation for the reaction velocity:

$$\frac{d \ln v_{\max.}}{dT} = \frac{A}{RT^2} \quad \text{or} \quad \frac{d \ln v_{\max.}}{d(1/T)} = -\frac{A}{R}, \qquad (11)^1$$

that is to say, the curve in the $\ln v_{\max.}$ by $1/T$ diagram ought to be rectilinear. A number of curves actually obtained are given in Fig. 3. They are taken from the work of Emerson & Green (1934) except for the curve of Warburg for *Chlorella pyrenoidosa* (Warburg, 1919).[2] It can be seen that at low temperatures there is a very steep slope which flattens as the temperature rises. The slopes at the ends of the curves correspond to the activation energies recorded at the respective points (as calculated by Emerson & Green for *their* curves). These quantities vary with temperature in a way incompatible with the theory of the unity of the reaction. The same is true of curves obtained by van der Paauw (1934) for three other

[1] A = activation energy, R = gas constant, T = absolute temperature.

[2] *Gigartina harveyana* and *Chlorella pyrenoidosa* have been investigated by Emerson & Green (1934), *Chorella vulgaris* by Emerson (1929*a*, *b*). The data for both species of *Chlorella* have been repeated with alterations which can be neglected here in the paper of Emerson & Green (1937). The experiments on *Hormidium flaccidum* are by van der Paauw (1932). Results later obtained by Yabusue (1924) in the institute of Warburg deviate slightly from those of Warburg in the sense of those of Emerson & Green.

green algae. A number of further temperature coefficients of photosynthesis have been collated or calculated from experimental figures by James (1934), who discusses in detail the abnormal variation of the coefficient.

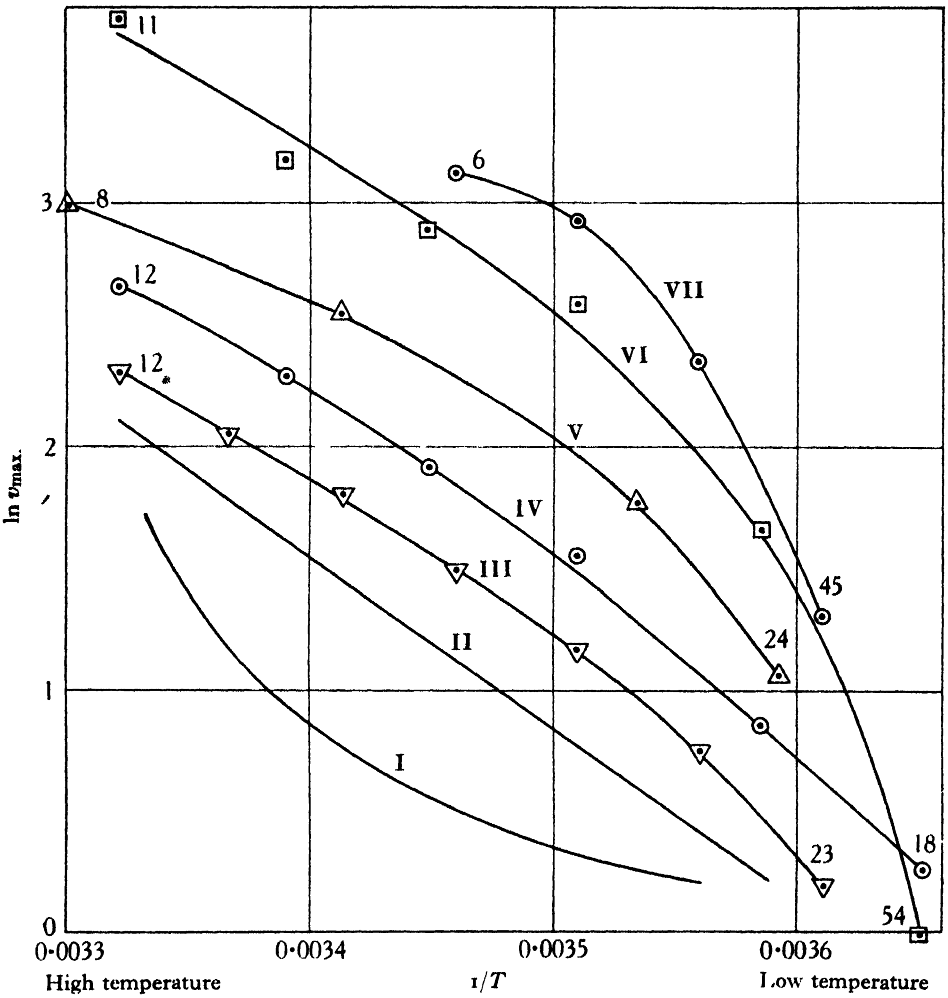

Fig. 3. I, conductivity of certain solid salts; II, a normal case; III, *Hormidium flaccidum*; IV, *Chlorella pyrenoidosa* (Emerson & Green); V, *Chlorella pyrenoidosa* (Warburg); VI, *Chlorella vulgaris*; VII, *Gigartina harveyana*. The recorded numbers are activation energies in k.cal./mole.

(ii) *The applicability of Arrhenius's equation to biological processes*

It is often thought that biological processes are too complicated to make possible the application of the Arrhenius equation in more than a purely formal sense. It is of course agreed that all velocity curves in biological systems fall away at very low temperatures abnormally steeply, and at high temperatures instead of continuously rising, they again fall back to low values. This is because the cell is damaged by extremes of temperature. (On the subject of high temperatures cf. Craig & Tre-

lease, 1937.) The abnormal behaviour at low temperature may be caused by changes in the colloidal state of the material which take place more or less discontinuously—as a blurred transformation of phases—while that at high temperature may be caused by side reactions which at these temperatures begin to obscure the main change. If such objections to the Arrhenius equation were generally valid then it would be expected that the curve of reaction velocity v would be of the form I in Fig. 4. In spite of this, however, biological processes generally show curves of form II (cf. the numerous papers of Crozier and co-workers in *J. gen. Physiol.*). This holds also for respiration (cf. e.g. Warburg (1925)). The curves for two of the four species of green algae investigated by van der Paauw (1934) are to my mind exceptional.

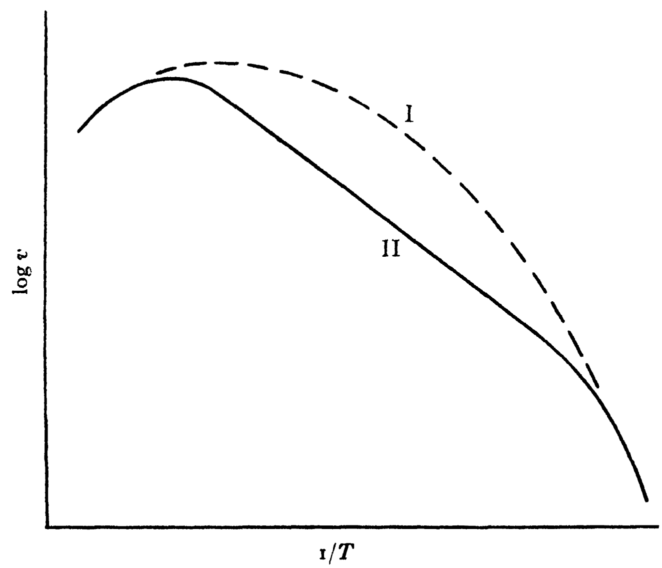

Fig. 4.

We are here, therefore, not concerned with the self-evident fact that in sufficiently narrow regions all continuous experimental curves appear to be linear within the limits of error, but with the observed validity of the simple Arrhenius equation in temperature regions favourable to biological activity which is so remarkable as to require some explanation.

It must be remembered, on the other hand, that according to Timoféeff *et al.* (1935; cf. also Jordan, 1938*b*) mutations originate in such a way that only one molecule of the gene is changed by one elementary act. Therefore the frequency of the occurrence of the unimaginably complicated process of the development of a visible variation, i.e. the velocity of the formation of mutations, is equal to the frequency of the simple change of one molecule whether it is produced by irradiation or by the effect of temperature. Therefore the frequency of mutation is governed by Arrhenius's law (Timoféeff *et al.* 1935).

Similarly, in other cases a single simple process, under given conditions and in limited regions, will control a complicated sequence of events. That this is often the case is shown by the frequent occurrence of curves of type II, Fig. 4. When therefore, a relatively self-contained process like that of photosynthesis deviates from the normal case in the manner shown in Fig. 3 then the deviation must have some special cause which we must attempt to explain as a variation from the normal.[1]

(iii) *The explanation of the abnormal shape of the assimilation/temperature curve*

Now cases occur in which the velocity curve in the $\ln v$ by $1/T$ diagram is bent in the contrary direction, as shown by the lowest curve in Fig. 3. Such cases are, for instance, the velocity of diffusion and the electric conductivity of certain solid salts. As is well known, this phenomenon can be explained by the fact that the process can proceed in two parallel ways, say by the diffusion of one or the other ion. Then the process which was the faster one at low temperatures will be exceeded by the other one at high temperatures, the latter having a higher activation energy and thus a greater temperature coefficient. But this explanation, as Emerson (1929*b*) has pointed out, cannot be transferred to curves which are convex as seen from the top.

The assimilation curves of Fig. 3 can be explained in the following way. The process of photosynthesis occurs at a given number of spots. Here all chemical reactions take place one after the other, and when the process is finished these spots again take up the initial substance. That is the reason why the mean periods of the partial reaction add up simply to the total Blackman period τ according to the equation $\tau = \tau_1 + \tau_2 \ldots$. If reactions follow each other the slowest one determines the velocity of the total process, and thus the reaction of higher activation energy giving the steeper slope is predominant at low temperatures.

It appears to be unnecessary to include in this discussion more reaction periods than two, τ_1 and τ_2. Following Emerson & Arnold (1932) and Pratt & Trelease (1938) we take the total Blackman period to be about 0·012 sec. at 25° C. in agreement with Table 1. Now it is possible to calculate absolute values of τ_1 and τ_2 at different temperatures from the observed data for $v_{\max}$. This can be done as follows, according to the equation of Arrhenius:

$$\tau_1 = \tau_1^{(0)} e^{A_1/RT}, \tag{12a}$$

$$\tau_2 = \tau_2^{(0)} e^{A_2/RT}, \tag{12b}$$

$$\tau = \tau_1 + \tau_2 = \tau_1^{(0)} e^{A_1/RT} + \tau_2^{(0)} e^{A_2/RT}. \tag{12c}$$

The constants $\tau_1^{(0)}$ and $\tau_2^{(0)}$ can be conceived as hypothetical reaction periods at infinitely high temperature. A_1 and A_2 are the activation energies. The values

[1] Yabusoe (1924) claimed to have demonstrated that the activity of catalase had the same abnormal temperature coefficient as assimilation. French (1934), however, failed to find any abnormal variation of catalase activity with temperature. This point was brought forward *inter alia* by Gaffron & Wohl (1936*a*) to show that the Blackman reaction is not a process of peroxide breakdown. Since the findings of Yabusoe concerning assimilation were not in doubt, Gaffron & Wohl felt it only necessary to cite that paper (French, 1934) in which his evidence on catalase was refuted. It seems advisable to make this statement, since Emerson & Green (1937) have so completely misunderstood the remark of Gaffron & Wohl on this subject.

calculated in this way for three of the experimental series of Fig. 3 are given in Table 2. Columns 2–5 show the constants of equation (12*c*), columns 8–13 show the magnitude of the total Blackman period $\tau = \tau_1 + \tau_2$ and the relative amount of τ_2 at two temperatures for each series. (The single values for τ_1 and τ_2 may thence easily be calculated.)

Table 2

1	2	3	4	5	6	7
	Constant factor $\tau_2^{(0)}$ sec.	Activation energy A_2 k.cal.	Constant factor $\tau_1^{(0)}$ sec.	Activa-ation energy A_1 k.cal.	Activa-ation entropy ΔS k.cal.	Concen-tration C mol./l.
Chlorella pyrenoidosa, Warburg	$10^{-5,6}$	5	$10^{-28,9}$	29	0·051	$10^{-3,3}$
Chlorella pyrenoidosa, Emerson & Green	$10^{-8,6}$	9	$10^{-19,1}$	23	0·029	$10^{1,3}$
Gigartina harveyana	$10^{-1,6}$	0	$10^{-46,6}$	58	0·154	$10^{-5,3}$

	8	9	10	11	12	13
	Temp. °C.	$\frac{\tau_2}{\tau_1+\tau_2}$	Total Blackman period $\tau_1+\tau_2$ sec.	Temp. °C.	$\frac{\tau_2}{\tau_1+\tau_2}$	Total Blackman period $\tau_1+\tau_2$ sec.
Chlorella pyrenoidosa, Warburg	25	0·849	$0{\cdot}013_5$	5	0·236	0·089
Chlorella pyrenoidosa, Emerson & Green	24·3	0·725	0·014	5	0·322	0·091
Gigartina harveyana	15	0·851	0·03	5	0·125	0·204

Cf. equations (12), (13) and (19). Columns 2, 3 and 7 refer to the bimolecular reaction, columns 4, 5 and 6 to the monomolecular one. τ_2 is the bimolecular Blackman period, τ_1 the monomolecular one.

Table 3

Chlorella pyrenoidosa, Warburg	Temp. °C.	5·4	10	20	30		
	v_{obs}	58	118	256	402		
	v_{calc}	58	107	256	402		
Chlorella pyrenoidosa, Emerson & Green	Temp. °C.	1	6	12	17	22	24
	v_{obs}	1	0·692	0·474	0·330	$0{\cdot}165_5$	$0{\cdot}091_4$
	v_{calc}	1	0·687	0·474	0·306	$0{\cdot}165_5$	$0{\cdot}091_4$
Gigartina harveyana	Temp. °C.	4	8	12	16		
	v_{obs}	11·3	34·9	66·9	82·6		
	v_{calc}	11·6	36·0	66·1	81·6		

Table 3 shows the close agreement between calculation and experimental data, which, however, is not surprising on account of the number of constants of equation (12*c*). The real test of the theory consists in the analysis of the absolute values of $\tau_1^{(0)}$ and $\tau_2^{(0)}$ in Table 2.

Table 2 indicates that the figures found by Emerson & Green and by Warburg for *Chlorella pyrenoidosa* are distinctly different, but when compared with those resulting for *Gigartina harveyana* they seem to coincide fairly well. According to

columns 9 and 12 of Table 2, τ_2 prevails at high temperature, whilst at low temperatures τ_1 is predominant. This is only possible, of course, if the constants $\tau_1^{(0)}$ and $\tau_2^{(0)}$ in equation (12*c*) compensate the difference of the *e*-functions caused by the different activation energies so that the products of both, i.e. the reaction periods τ_1 and τ_2, have the same value at a medium temperature. Therefore $\tau_1^{(0)}$ belonging to a reaction with a high activation energy has to be several orders of magnitude smaller than $\tau_2^{(0)}$ which is coupled with a low activation energy. So it follows that the two reactions are of quite different types.

(iv) *The bimolecular and the abnormal monomolecular Blackman reaction*

Now the absolute values of the constants $\tau_1^{(0)}$ and $\tau_2^{(0)}$ reveal at once that $\tau_1^{(0)}$ relates to a monomolecular, $\tau_2^{(0)}$ to a bimolecular reaction. This may be shown in the first instance for $\tau_2^{(0)}$. The reciprocal of τ_2 is the reaction velocity or the number of *successful* collisions per second per reducing centre. Then $1/\tau_2^{(0)}$ is essentially the collision number at one reducing centre, and therefore we have

$$\frac{1}{\tau_2^{(0)}} = k_2^{(0)}c. \tag{13}$$

Here c is the concentration of the reacting partner in mol. per litre and $k_2^{(0)}$ the collision constant, i.e. the number of collisions at a concentration of 1 mol. per litre. If we choose a rather small molecule as a reacting partner, say of molecular weight of 130 and with one reactive group, we may estimate from the usual formula for gaseous systems the upper limit of $k_2^{(0)}$ to be $10^{9\cdot 9}$. If the reaction has practically no activation energy, as in the case of *Gigartina harveyana*, then when the reaction occurs in a solution, this constant, on account of the dissolved molecules being impeded by the solvent, is lessened by about 1/1000, i.e. to about $10^{6\cdot 9}$. With an activation energy of 5 kcal., as in the case of Warburg's experiment, it may be assumed that the constant is still reduced by a certain amount, while in the case of an activation energy of 9 kcal. (Emerson & Green's data for *Chlorella pyrenoidosa*), the constant has the full magnitude valid for gaseous systems. Now equation (13) makes it possible to deduce from the collision number $1/\tau_2^{(0)}$ and the collision constant $k_2^{(0)}$ the concentration of the reacting partner. Taking into account the variability of the constants with varying activation energy, it is found according to column 7 of Table 2 that the lower limit of concentration lies between 0·04 and 4×10^{-6} mol. per litre, which may be considered reasonable values.

This calculation at the same time makes it clear that the values of $1/\tau_1^{(0)}$ (cf. column 4 of Table 2) are too high to be ascribed to a collision reaction. So they relate to a monomolecular reaction. Then $1/\tau_1$ is a monomolecular velocity constant k_1. In this type of reaction the number of opportunities of reacting per sec. is approximately given by the number of vibrations of the surrounding atoms or groups. At room temperature this is about $10^{12\cdot 8}$.

So it would be expected that the constant $1/\tau_1^{(0)}$ of equation (12*a*), which may be restated in the form

$$\frac{1}{\tau_1} = k_1 = \frac{1}{\tau_1^{(0)}} e^{-A_1/RT}, \tag{14}$$

has the value $10^{12\cdot 8}$ instead of those registered in Table 2.

Now it is well known that $1/\tau_1^{(0)}$ equals the theoretical value only very roughly, and in principle we know the reason. For this equation should contain, instead of the activation energy, only the necessary supply of *free* energy ΔF, that is to say the amount of energy which an elementary act such as a collision between atoms or the absorption of a light quantum must transfer to the system in order that the reaction may be brought about. But on this occasion additional amounts of energy are swiftly exchanged with the surroundings. They join the free energy and supplement it to form the total activation energy A_1 which appears in the equation for the temperature coefficient. These amounts of energy, given by the product of the temperature T and the entropy difference ΔS, will be considerable, especially if the freedom of movement of the system is changed by activation. They have positive values, i.e. they are *conveyed to* the system *from* its surroundings and thus support the effect of the free energy in the event of freedom of movement being increased, as, for example, if during activation the bond between two atomic groups is loosened so that these are able to rotate.

The more exact equation for the absolute value of the velocity constant of the reaction is thus

$$\frac{1}{\tau_1} = k_1 = 10^{12 \cdot 8} \times 10^{-\frac{\Delta F}{2 \cdot 303 RT}}. \tag{15}$$

Further, the variation of the constant with temperature, according to equation (11), is given by

$$\frac{d \ln k_1}{d(1/T)} = -\frac{A_1}{R}. \tag{16}$$

Finally, both energy quantities are connected by the thermodynamic equation

$$\Delta F + T\Delta S = A_1. \tag{17}$$

If we now introduce the value of A_1 derived from the equation (16) with the help of equation (17) into the equation (15) we get

$$\frac{1}{\tau_1} = k_1 = 10^{12 \cdot 8} \times 10^{-\frac{(A_1 - T\Delta S)}{2 \cdot 303 RT}} = 10^{12 \cdot 8 + \frac{\Delta S}{2 \cdot 303 R}} \times 10^{-\frac{A_1}{2 \cdot 303 RT}} = \frac{1}{\tau_1^{(0)}} \times 10^{-\frac{A_1}{2 \cdot 303 RT}}. \tag{18}$$

Thus it follows

$$\frac{1}{\tau_1^{(0)}} = 10^{12 \cdot 8 + \frac{\Delta S}{2 \cdot 303 R}}, \tag{19}$$

i.e. $1/\tau_1^{(0)}$, in the case of positive values of ΔS, actually becomes bigger than $10^{12 \cdot 8}$. The values derived for ΔS from equations (19) are given in column 6 of Table 2.

(v) *The direct production of sugar at the reducing centre and the transition of oxygen to a dissolved molecule*

The entropy factor generally alters the normal factor $10^{12 \cdot 8}$ (equations (18) and (19)) only by a few powers of 10; accordingly ΔS generally keeps within the limits $\pm 0 \cdot 010$ kcal. Indeed, considerably larger effects cannot theoretically be expected so long as only one or two atomic groups are involved in the chemical change. The abnormally high values of the constant factor $1/\tau_1^{(0)}$, and so of the activation entropy ΔS, of a monomolecular reaction can be explained by assuming that in this reaction a greater number of bonds are broken and that the separated groups are

allowed to rotate (see e.g. Stearn & Eyring, 1937). We thus see that the monomolecular Blackman reaction cannot simply consist in the separation of an oxygen molecule or a formaldehyde molecule from the reducing centre.

It might be considered that there is engaged in photosynthesis an auxiliary substance which is normally attached to the reducing centre and its neighbourhood with a greater number of bonds and which becomes loosened with all its bonds in the monomolecular Blackman reaction. Subsequently, of course, the substance would have to be again spontaneously attached in order to fulfil its task in the next photosynthetic cycle. A process of this kind, however, does not seem to be likely.

It would be simplest if these anomalies could be explained by the action of one of the photosynthetic products itself without having recourse to the process mentioned. And this would appear possible, since the idea that by the Blackman reaction formaldehyde is liberated from the reducing centre is an hypothesis which cannot be substantiated. The first tangible product of photosynthesis is a sugar, either glucose or fructose or sucrose. So we have to assume that one of these substances is formed directly at the reducing centre and is detached in its entirety in the monomolecular reaction. Thus, in the case of a hexose, six bonds would be simultaneously broken, and twelve atomic groups allowed to rotate more or less freely.

The question how a hexose molecule is formed at the reducing centre and kept there, may, to begin with, be answered by pointing out how it could *not* be done: it is impossible for six formaldehyde groups to be formed independently out of carbon dioxide at six adjacent places, and for these subsequently to join to form glucose and leave together. It can be shown that such a process would make necessary at least 6·7 quanta for the reduction of one carbon dioxide molecule whereas Warburg & Negelein (1923) and Rieke (1939) experimentally found that 4·2 quanta sufficed.

Thus the formaldehyde groups must be formed one after the other at the same reducing centre, and must be transferred successively to neighbouring groups until six groups form one glucose molecule which leaves the reducing centre in the Blackman reaction. In this way photosynthesis would be a 24-quanta process: after every four quanta one oxygen molecule is delivered and one carbon dioxide molecule attached. After six processes of this kind the glucose molecule becomes loosened by a monomolecular reaction, the mean period of which is six times longer than the reaction period τ_1 following from Table 2. The mechanism seems to be in accord with all the other facts of assimilation just as is the mechanism dealing with formaldehyde as the final product of photosynthesis (Wohl, 1937*d*).

As to the nature of the neighbouring groups we may perhaps imagine that they might be protein groups able to bind the hydroxyl groups of the sugar by hydrogen bonds. In this way our monomolecular reaction would be an analogue of the findings of Stearn & Eyring (1937) who explained the abnormal variation of the denaturation velocity of albumin molecules with temperature by the simultaneous breaking up of several hydrogen bonds.

An approximate idea of the situation can be formed by distributing the total activation entropies given in Table 2 among the number of atomic groups supposed to become loosened and by testing whether the single amounts of entropy are

compatible with values which can be assumed for the degrees of freedom and moments of inertia of the groups. In this place it may only be said that according to this test the theory seems to be possible (for *Chlorella pyrenoidosa* cf. Wohl, 1937*c*) and that in the case of *Gigartina harveyana* and *Chlorella vulgaris* a direct formation of sucrose, instead of hexose, might be taken into consideration.

It seems possible that certain types of experiments with flashing light may decide the problem of whether the sugar is or is not detached as a whole.[1]

From our view that the monomolecular reaction consists in the detaching of the carbohydrate it follows naturally that the bimolecular reaction is the formation of oxygen. This we may imagine to occur in the following way. Some dissolved substance (i.e. an unfixed enzyme), by a collision reaction with a small activation energy, takes over the oxygen from the reducing centre and after some time sets it free.[2] The writer feels that recent experiments of Gaffron (1939) on the influence of hydrogen on photosynthesis support this view.

(vi) *The explanation of the shape of the assimilation curve proposed by Smith*

The mechanism proposed for the development of oxygen furnishes a simple explanation of the observations of Smith (1938) according to which the shape of the curves showing the dependence of photosynthesis on light intensity and carbon dioxide concentration does not correspond to the usual and simplest theoretical statement (cf. Burk & Lineweaver, 1935; Gaffron & Wohl, 1936*a*; Wohl, 1937*a*). (The writer is, however, of the opinion that the deviation from the simplest shape of the curve is not so clear in a number of cases as Smith suggests.) The form of Smith's assimilation curve corresponds to a lengthening of the Blackman period if light intensity or carbon dioxide concentration or both are increased, i.e. if the velocity of assimilation rises (Wohl, 1937*c*). This can come about if one of the products of photosynthesis is able to react reversely with the reducing centre. As the temperature in the experiments of Smith was as high as 25° C. the product in question will be the enzyme charged with oxygen as at high temperatures the bimolecular reaction essentially determines the rate of assimilation. For the development of oxygen will be the more retarded the oftener the reverse reaction takes place.

[1] There would appear to be yet another possible physico-chemical model which is able to account for a big change of activation energy in a small range of temperature. The modification of the kinetic mechanism of the photosynthetic unit mentioned on p. 47 makes it possible to apply this model to photosynthesis. The abnormal change of activation energy in the case of *Gigartina* (Table 2), however, seems to be beyond the possibilities of this model, unless additional assumptions are made. For further information on this model see Wohl (1937*d*, *e*).

[2] In agreement with Gaffron & Wohl (1936*a*), Gaffron (1937) and Emerson & Green (1937) the writer believes that no true peroxide is formed in photosynthesis, i.e. no highly oxidative compound with one active oxygen atom. In Wohl (1935) it is shown that the mechanisms proposed by Stoll (1932), Willstätter (1933) and Franck (1935) and those proposed by Willstätter & Stoll (1918, p. 242) and Warburg (1928, p. 425) are impossible for energetic reasons, the former on account of the inclusion of hydrogen peroxide as an hypothetical intermediate, the latter on account of the similar inclusion of performic acid. Now Emerson & Green (1937) state concerning these calculations that "no definite claim has been made that the peroxide formed must be hydrogen peroxide". This comment is incorrect. The calculation was made in order to refute Franck's theory which has been described in detail, e.g. in Emerson's review (1936) and in which hydrogen peroxide cannot be substituted by another peroxide (cf. Franck & Herzfeld, 1937*a*).

This reverse reaction will be most frequent when the stationary concentration of the enzyme charged with oxygen is high. This is governed by the ease with which combination of the enzyme with oxygen can occur, and this in its turn by the frequency with which oxygen is produced at the reducing centre.

The observation of Smith (1938) can also be explained by postulating a mechanism quite different from the reversible enzyme reaction described above, viz. that suggested by Briggs (1935) and Burk & Lineweaver (1935) who assume that the amount of enzyme is commensurate with the number of reducing centres. The writer feels, however, that the plausibility of this assumption is diminished if the theory of the photosynthetic unit is accepted because in this theory the number of reducing centres is very much smaller than had been supposed by the authors mentioned. To decide between these two alternatives it would be useful to examine the shape of the curve at low temperature (cf. Wohl, 1937*c*).

This communication has been almost completely restricted to the consideration of assimilation in a steady state and in conditions of carbon dioxide excess. Space will not permit discussion of the condition in which carbon dioxide becomes the limiting factor or of the phenomena of the induction period. A kinetic treatment of the former problem may be found in the papers of James (1934), Briggs (1933, 1935), Burk & Lineweaver (1935) and Brackett (1935), and a detailed discussion is given by Emerson (1936). Recent experimental results and further useful discussions have been published by James (1928), van den Honert (1930), van der Paauw (1932), Hoover *et al.* (1933), Arens (1936*a*, *b*), Emerson & Green (1938), Smith (1938) and others. In addition to the papers on induction period already noted (Warburg, 1920; McAlister, 1937; Smith, 1937) mention must be made of those by Gaffron (1935*b*, 1937) which include a treatment of the problem from a chemical point of view.

ADDITION IN PROOF

Recently, J. Franck has revised his view (Franck and Herzfeld, 1937*b*) about the "optical model" of a photochemical unit consisting of a great number of molecules which Gaffron and Wohl established in 1936. He has, however, not made clear this situation. This model was found to be probably valid in the case of polymerized pseudocynanines by G. Scheibe (*Naturwissenschaften*, **25**, 795, 1937 and *Kolloidzschr.* **82**, 1, 1938) and in the case of phosphorescent substances by N. Riehl (*Ann. Phys., Lpz.*, **29**, 640, 1937 and *Z. angew. Chem.* **51**, 300, 1938), as has been mentioned in this paper (Section B viii). Now J. Franck and E. Teller (*J. Chem. Phys.* **6**, 861, 1938) subject this model to a detailed theoretical discussion. They come to the conclusion that the realization of the model in its original field of application, i.e. in the case of photosynthesis, is doubtful, and can be decided definitely by a fluorescence experiment which has still to be carried out. Yet they find that this mechanism probably exists in the case of pseudo-cyanines, though not in the case of phosphorescent substances.

The situation has also not been made clear in J. Franck, *J. Wash. Acad. Science*, **27**, 317, 1937. This paper deals with the relation of the photosynthetic unit to the phenomena at low light intensity and with the calculation of the functional photosynthetic unit from the assimilation/light intensity curve communicated by Gaffron and Wohl (1936*a*) (cf. this paper, Sections B i and iii).

Lately S. E. Sheppard, R. H. Lambert and R. D. Walker (*J. Chem. Phys.* **7**, 426, 1939) suggest that a mechanism like that of the optical model of the photosynthetic unit may exist in the case of sensitization of the photographic plate. (Like Franck and Teller, however, they challenge the accumulation of quanta suggested by Scheibe (1937).)

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MECHANICAL STIMULATION AND RESPIRATION IN THE GREEN LEAF

III. THE EFFECT OF STIMULATION ON THE RATE OF FERMENTATION

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(With 5 figures in the text)

Introduction

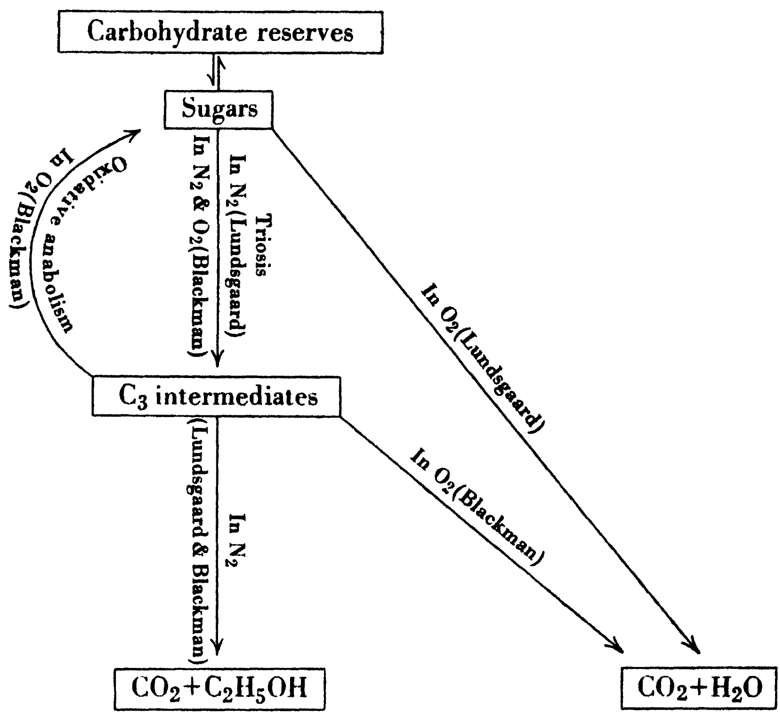
In two previous papers (Audus, 1935, 1939) the author has shown that the respiratory system of the starving green leaf is extremely sensitive to mechanical deformation of the leaf cells, the constant feature of the response to such stimulation being the sudden rise in the respiration rate to about twice the normal value and the gradual recovery over a period of about 48 hr. Many attempts have been made by earlier workers to elucidate the physiological causes underlying the huge respiratory responses observed in such traumatic stimulations as the cutting of storage tissues, a process not only involving cell-deformation responses but also a complexity of effects of cell injury. In this respect the relationships with oxygen concentration were principally investigated (Boehm, 1887; Smirnoff, 1903; Stich, 1891; Tscherniajew, 1902). The conclusions drawn were that the large increase in respiration rate after cutting was due entirely to the increased metabolism of wound healing, which apparently does not take place in the absence of oxygen.

It would seem that these experiments were premature since very little was known at this time concerning the interrelationships of fermentation and oxidative respiration in tissues of the higher plants and of the complex adjustments that take place in passing from aerobic to anaerobic conditions and vice versa. Since then the work of F. F. Blackman and his school has done much to elucidate these relationships. It is now widely accepted that the two processes are intimately connected, the initial stages of the breakdown of sugars into C_3 intermediates being common to both, although the animal biochemist, Lundsgaard, holds that they are completely independent. The present state of our knowledge of these two processes and of theories concerning their relationships in the higher plant is admirably summed up by Turner (1937). The broad outline of the two theories are illustrated in the schema on p. 66.

Bearing these two possibilities in mind, we turn to the problem of the causes underlying the increased respiration of the leaf after cell deformation. We see that investigations on the effects of stimulation upon the fermentation of the leaf provide us with a possible method of determining the locus of action of this stimulation in the respiratory sequence of reactions. Thus if cell deformation results in any stimulation

of reserve hydrolysis, then we should expect the CO_2 output in nitrogen to be affected in the same way as the normal CO_2 output in air. On the other hand, a definite absence of response in nitrogen would indicate that stimulation acts specifically on the oxidative stages. These conclusions would be valid on either theory. On the Blackman hypothesis, however, an absence of effect on fermentation would localize the action more specifically to the post-triosis stages.

With these possibilities in view, experiments were designed to investigate the effects on the CO_2 output of cherry laurel leaves of stimulations in pure nitrogen and low concentrations of oxygen.



Experimental methods

The cherry laurel material was from the same source as that used in earlier experiments (Audus, 1935). The leaves taken were as uniform morphologically as possible and were all of the current year. Continuous three-hourly readings of the respiration at 22·5° C. were made by the pettenkofer technique.

The nitrogen was prepared by passing commercial cylinder nitrogen (containing anything from 0·5 to 0·9% of oxygen) over copper gauze in an electric furnace at approximately 400° C. The nitrogen issuing from the furnace contained no oxygen detectable by the ordinary methods of gas analysis. It was led to the respiration chamber after passing through a cooling coil and soda lime tower.

Results of a number of preliminary experiments showed that simple transverse bending of the leaf lamina evoked a respiratory response almost as great as the stimulation process carried out by hand (see previous paper, Audus, 1935), and therefore a chamber was designed whereby the leaf sample could be subjected to bending without opening the chamber or disturbing the gas current in any way. The

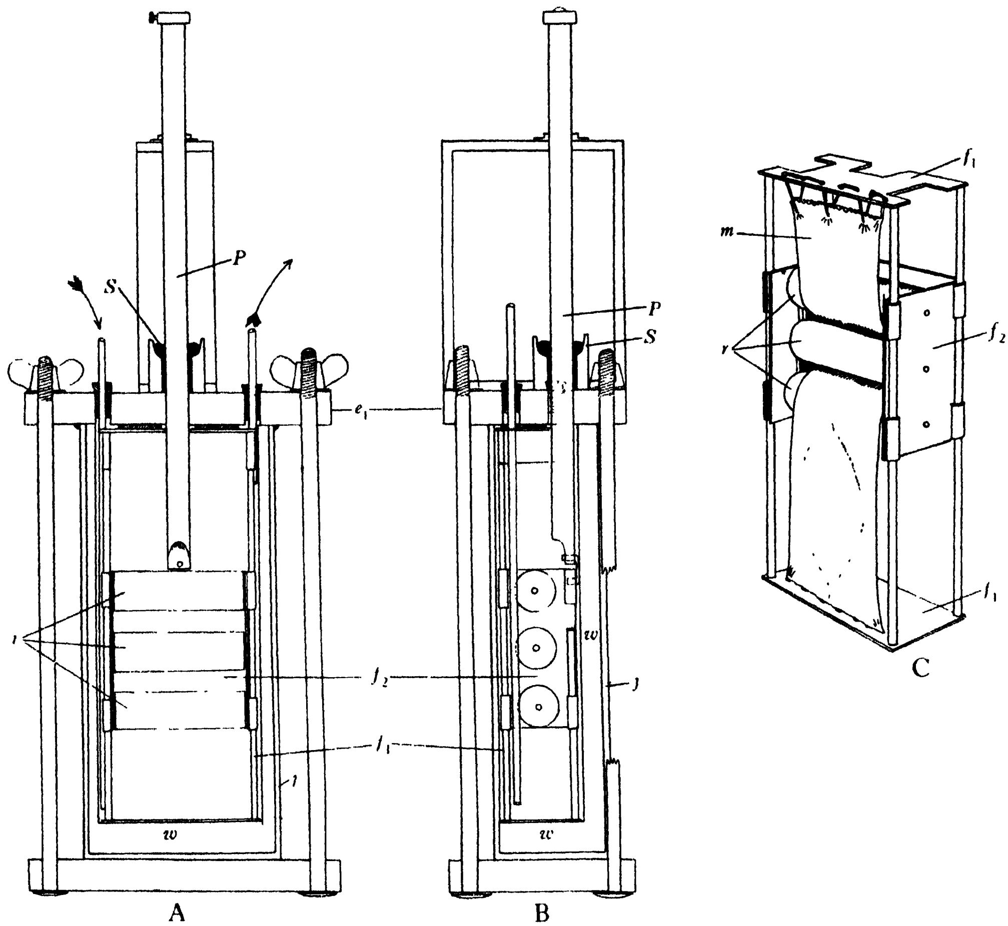


Fig. 1. Illustrations of chamber for the bending of a sample of leaves in nitrogen and in low concentrations of oxygen. A. Front elevation. B. Side elevation. C. Drawing of roller mechanism for effecting the stimulation of the sample. (For further description see text.)

final apparatus evolved is illustrated in Fig. 1. The arrangement used for bending the leaves is seen on the right of the figure and consisted of a brass frame (f_1) possessing four parallel brass runners on which ran a second brass frame (f_2) carrying three oak rollers (r). To the bottom of the main frame was sown a muslin bag (m), which passed upwards between the rollers and was attached by a string to the top of the frame. A string ran up the edge of the bag to keep it taut transversely.

When the bag had been fastened in position the bottom of the frame was dipped into molten paraffin wax to strengthen the joint of bag to plate. The leaf sample was placed petioles downward in the bag, which was then sown tightly to the top of the frame (f_1). The leaves were kept in position in the bottom of the bag by sewing the muslin together around them. The bag was of such a height that when the rollers were drawn to the top of the main frame the leaves were free and undeformed. By pushing the roller-frame up and down the main frame the bag and leaves contained in it were bent around the rollers and the leaves stimulated. The leaves were not damaged in any way by this treatment.

The chamber itself consisted of a rectangular museum jar (j) rather larger than the main frame and made to fit it exactly by lining with paraffin wax (w) along three sides and the bottom to reduce the volume of the chamber to a minimum. The jar was provided with a heavy ebonite lid through which passed a steel plunger (P) working in a steel guide. This plunger was fixed by a hook to the movable frame which could thus be moved up and down along its runners. A watertight seal was made between chamber and lid by means of low melting-point luting wax and between plunger and lid by the mercury seal (S). The water supply to the leaves was run into the bottom of the chamber so that the cut ends of the petioles were just covered.

In experiments with this chamber, samples of six leaves were used and the respiration followed with the plunger up so that the leaves were not bent. Stimulation was effected by depressing and raising the plunger five times. This technique was found to give a stimulation effect identical with those produced by hand.

Two leaf samples were chosen and the starvation respiration of both followed, one control sample in the simple respiration chamber previously described (Audus, 1935) and the other in the special stimulation chamber. It should be noted here that the latter sample consisted of only six leaves whereas the control sample was of fifteen. The readings given by the small sample showed greater random fluctuations and hence less smooth curves than the larger sample. Both samples were given nitrogen at the same time and for the same period. The sample in the special chamber was stimulated, not only in nitrogen but also in air either before or after the nitrogen period. By this means an estimate was obtained of the stimulation effect in air with which to compare the effect in nitrogen. The necessity for the establishment of a standard of this kind will be apparent when it is remembered that the size of the effect varies considerably from sample to sample, although series of effects on the same sample show no such large random fluctuations. Gas transitions and stimulations were confined to the protoplasmic phase of the starvation respiration drift, for it is in this region of the curve that the most uniform series of effects was obtained in air.

RESULTS

Experiment indicates that the respiratory behaviour of cherry laurel leaves in nitrogen is similar to that of the apple as shown by Parija (1928). Thus in Fig. 2 the result of a typical experiment is illustrated. The smooth double curve shows the

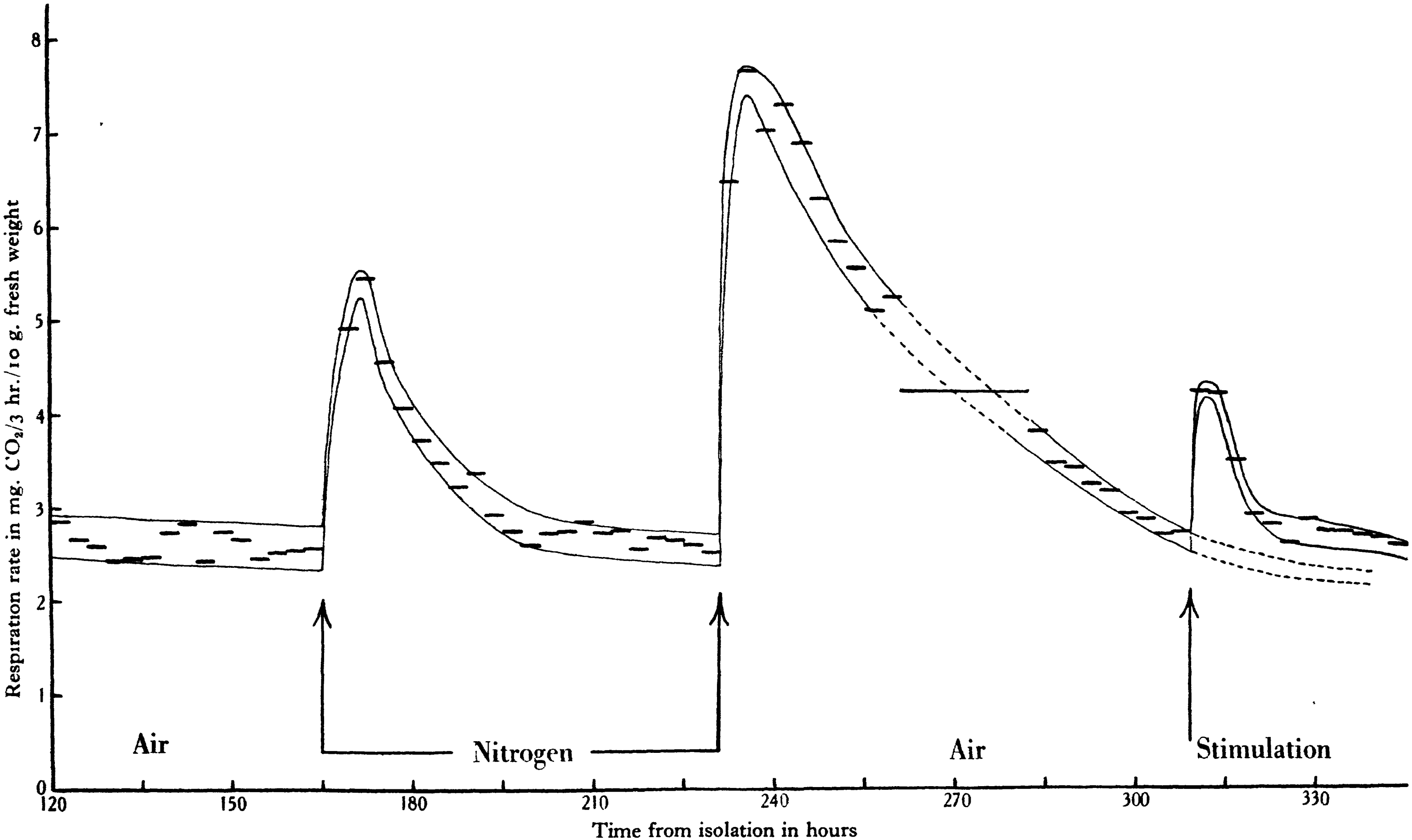


Fig. 2. Graph showing the respiratory behaviour of cherry laurel leaves during and subsequent to a period of 66 hr. in nitrogen. A normal stimulation effect in air after the nitrogen experience is seen at 309 hr. from isolation.

respiration drift during starvation with the effects on the CO_2 output of an interpolated nitrogen period, which is marked by arrows. It will be observed that after transference to nitrogen there is an immediate rise in the rate of CO_2 output by the leaves. This is due to the sudden diversion of the products of sugar triosis from oxidative respiration to fermentation and, on the Blackman theory of respiration, would indicate a high ratio of oxidative anabolism to oxidation in air. From this initial high value the rate of CO_2 evolution falls away as the slow adjustment of the triosis rate takes place under the new conditions, and eventually reaches a steady value, which, in this case, coincides with the normal drift rate in air. After longer periods in nitrogen the rate may again descend rapidly, but in the experiments under discussion these long periods were never used. Stimulations were carried out when the steady value of CO_2 evolution in nitrogen had been reached. The greatly increased respiration after nitrogen is due to oxidation of alcohol produced in fermentation.

In Fig. 3 the results of three such experiments have been brought together. The relevant portions of the respiration curves have been drawn, including a portion of the drift in air before the transition. These graphs show quite conclusively that stimulation had no significant effect on the rate of fermentation. On the Blackman theory of respiration this would therefore suggest that stimulation were acting on one or more of the oxidative respiration stages subsequent to triosis. It is a possibility, however, that the nitrogen destroyed the capacity of the cell to react to stimulation, both in nitrogen and in air subsequently. In this case the above conclusions would not be valid. Experiment shows, however, that the cell completely recovers its sensitivity to stimulation on returning to air. This is clearly demonstrated in the graph of Fig. 2.

In addition to experiments in pure nitrogen stimulations have also been carried out in low concentrations of oxygen. Under these conditions the CO_2 evolved comes partly from fermentation and partly from oxidation of the respiratory substrates, the ratio of oxidative respiration to fermentation rising with the oxygen concentration. From the foregoing results in pure nitrogen it would be expected that stimulations in these low concentrations of oxygen would result in definite but small effects from the reaction of the oxidative component of the gross respiration.

The two concentrations of oxygen used were 0·9 and 2·5 %, the other component of the mixtures being nitrogen. Experiments were carried out in the same way as those with nitrogen. Periods of the gas mixtures were given to leaf samples during the protoplasmic stage of their starvation life, and stimulations carried out in the mixtures when steady rates of CO_2 evolution had been attained after the initial transition effects. The results of three experiments are shown in Fig. 4, where the relevant smooth respiration curves have been drawn. It will be seen that the expectations are borne out, there being small but quite significant stimulation effects in these low concentrations of oxygen. Scanty as these results are, there seems to be a definite relationship between the size of the effect and the oxygen concentration. In all these experiments stimulations were carried out in air on the

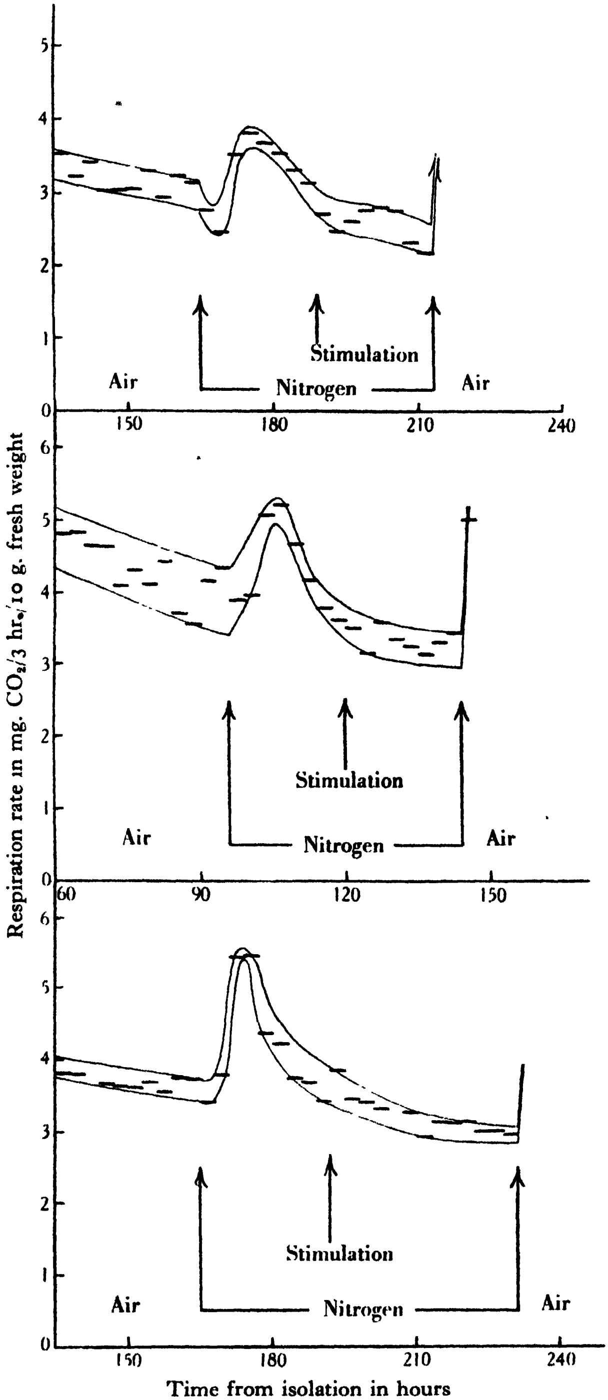


Fig. 3. Graphs showing the absence of any effect of stimulation on the rate of CO_2 output of cherry laurel leaves in nitrogen.

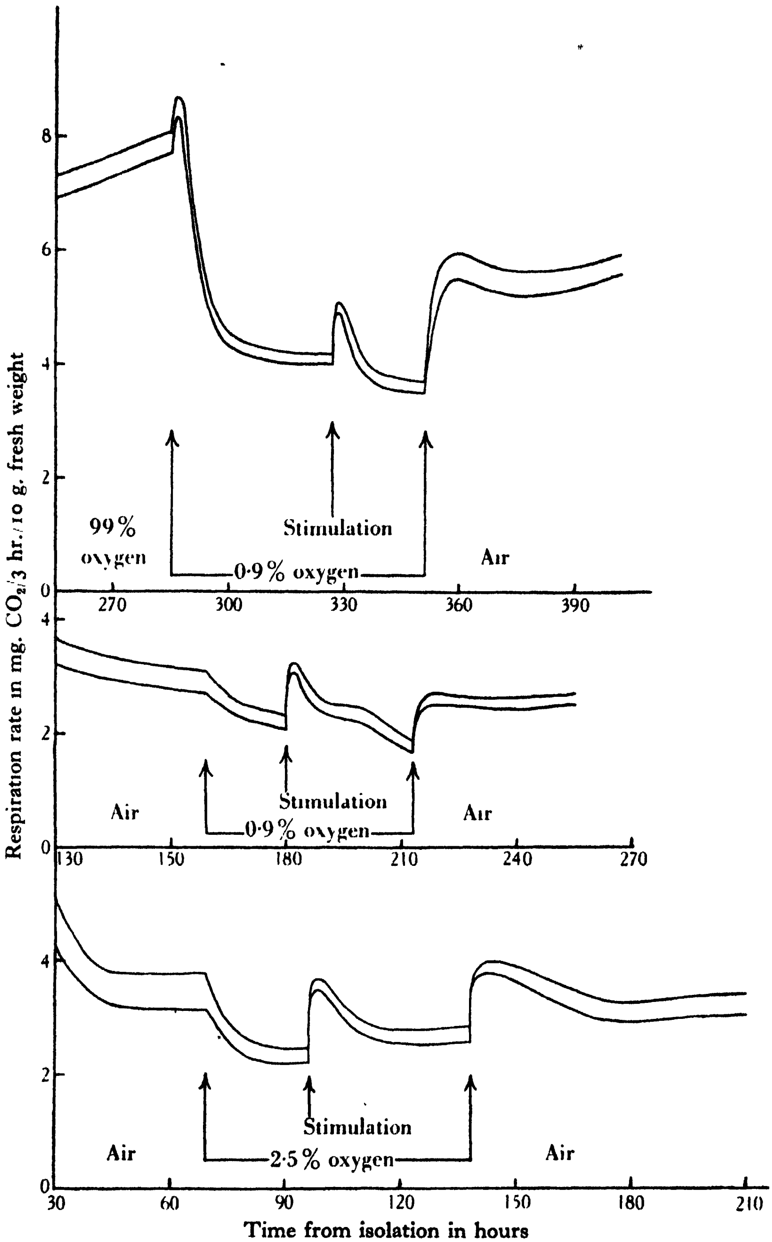


Fig. 4. Smoothed respiration curves of experiments on three leaf samples in low oxygen concentrations, showing small but significant stimulation effects. The two curves mark the vertical range of individual observations.

same leaf sample and the ratio of the stimulation rise (height of peak of effect above unstimulated rate) in the gas mixture to that in air has been determined in all three cases. These ratios, together with the zero values for the results in nitrogen, have been plotted against oxygen concentration in Fig. 5. A freehand curve drawn through the points shows the sudden rise of the effect from zero in nitrogen as the oxygen concentration increases. This rise presumably runs parallel to the increasing proportion of the oxidative component of the gross respiration.

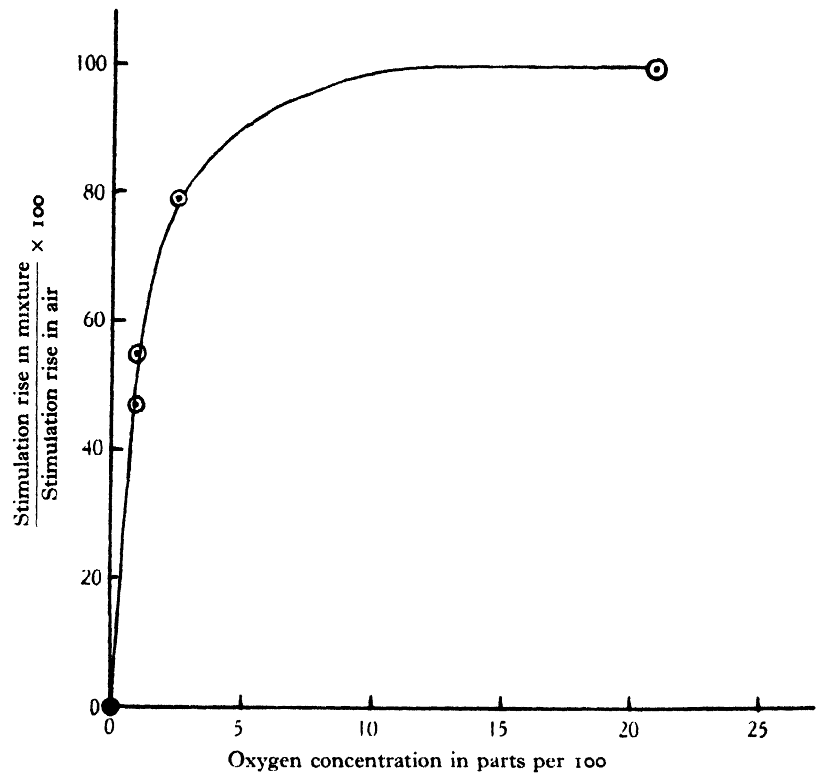


Fig. 5. Graph illustrating the relationship between oxygen concentration and magnitude of stimulation effect relative to that in air. The smooth curve is freehand.

CONCLUSIONS

The two sets of experiments which have been described have shown that in nitrogen and probably in low concentrations of oxygen stimulation of leaves has no effect upon the rate of fermentation, nor does this treatment affect the subsequent sensitivity to stimulation in air. The results suggest that stimulation does not affect the normal respiration rate through the provision of respiratory substrates by hydrolysis of reserves, or, on the Blackman hypothesis, by an augmentation of the rate of sugar triosis. Its action must, therefore, be on the oxidation process itself. A change in the physical organization of the cell resulting in a greater mobility of respiratory carriers or an increased permeability to oxygen are possibilities. Obviously further

advance along these lines is bound up with the whole question of the relationships between oxidative respiration and fermentation in low oxygen concentrations, and therefore this present aspect of the problem concerning the exact nature of the stimulation effect must await detailed investigations of such relationships.

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LYSIGENOUS AIR SPACES IN THE LEAF OF LABRADOR TEA, *LEDUM GROENLANDICUM* OEDER.

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(With Plate 1)

LABRADOR TEA, *Ledum groenlandicum* Oeder., an evergreen, ericaceous, bog plant, exhibits an interesting combination of so-called xeromorphic characters with others ordinarily considered as characteristic only of hydrophytes.

A number of these characters are to be seen in Pl. 1, fig. 2, from a transverse section through a mature leaf, stained only with Sudan III and mounted in glycerin. The prominent cuticle covering the outer walls of the upper and lower epidermal cells is brought out clearly by the stain, as are also the heavily cuticularized hairs, which are plentiful on the dorsal surface. Where the inner surfaces of epidermal cells are in contact with air spaces, they also have a cuticle, which appears in the photograph as a black line, less heavy than that on the outer surface. In this figure may also be seen the regular, rather closely packed palisade and mesophyll cells of the leathery, ericoid leaf.

In contrast with these xeromorphic structures are the prominent and plentiful lacunae which account for a very considerable part of the leaf volume. Such air spaces are often found in water plants, or in submerged parts of forms normally growing on land, but their abundance in a leaf the structure of which is otherwise that of a xerophyte requires some explanation.

It will be seen in the photograph that the lacunae alternate with small veins of the leaf. These veins form a fine network, and every islet of mesophyll tissue has one lacuna separated from the veins on all sides by one or more layers of the normal, small, closely packed mesophyll cells. The lacunae are clearly lysigenous, the remains of broken-down cells being visible at several points. In the mature leaf they are of such a size that the dorsal epidermis has been pushed out into hemispherical mounds, and a similar though less pronounced bulging is discernible on the ventral side.

Morphological and physiological investigations of the ontogeny of lysigenous spaces in the roots of Indian corn have been described by McPherson (1939) in a recent paper. He concludes that in this plant their development is similar to that in the roots of water plants investigated by Stover (1928–9) and Severin (1932), and is brought about by anaerobic respiration. He was able to prevent their occurrence whether the roots were immersed in water or not, by increasing the oxygen pressure, and to increase their extent greatly by causing the roots to grow in unaerated water, or with limited oxygen supply. He suggested that in the latter case anaerobic respiration took place in the cortical cells of the root, leading to an accelerated

destruction of food materials, as postulated by Blackman (1928), and a resultant condition of starvation in which the cell proteins were broken down by respiration.

The morphological changes leading to the formation of lacunae in *Ledum* leaves are indicated in the photographs. Pl. 1, fig. 1, is from a transverse section of a young leaf in a winter bud collected 1 November. Differentiation in the midrib has proceeded to some extent, and the elongated, narrow cells to the left indicate the beginning of a vein branching off from it. There is at this stage no evidence of the beginning of air spaces in any part of the leaf, and no difference can be detected between the cells that will later disappear and the rest of the mesophyll cells.

No further morphological change occurs until spring, when the buds swell, and rapid leaf development begins. Some time after the beginning of this rapid expansion the first sign of developing air spaces appears. Pl. 1, fig. 3 shows an early stage found in the leaf of an opening bud collected 21 May. On the bottom of the figure is a developing vein, and the group of small cells at the top indicates an early stage in the development of a younger one. Midway between the veins is a group of cells that have increased in size quite considerably by vacuolation. Cells such as these disappear at a later stage to form the lacuna. The group of actively vacuolating cells is completely surrounded by mesophyll cells not undergoing this rapid enlargement.

Pl. 1, fig. 4, shows a somewhat later stage. A few of the cells have become very large and a further modification has developed. The protoplasm in these swollen cells appears for the most part to have disappeared, or at any rate to have lost both its staining and its osmotic properties. The loss of semi-permeability is shown by the fact that the largest cells have not only ceased to expand, but are no longer turgid. Their walls are pushed in here and there by the turgidity of the smaller adjacent cells, and in one place, marked by the arrow, the wall of one of them has collapsed away from the other cells, leaving a small, irregular space. This series of events, namely excessive vacuolation followed by loss of osmotic properties and final complete disintegration of the protoplasm, continues until the large spaces shown in Pl. 1, fig. 2, result.

The next step toward a proper understanding of the spaces would appear to be a search for the causal factors that account for the pronounced vacuolation of these particular cells. This is especially necessary since the water they have absorbed must have passed to them from the veins through intervening layers of normal cells.

Three possibilities present themselves:

(1) The relatively greater suction pressure of the vacuolating cells may arise not from any difference in osmotic properties, but from cell wall differences. The walls of the vacuolating cells may have remained soft and easily extensible while those of the surrounding ones hardened.

(2) The osmotic pressure of the sap in the rapidly vacuolating cells may have increased above that of the cells between them and the veins.

(3) Permeability to water on the part of the protoplasm of the central cells may have increased while that of cells adjacent to the veins did not. On this hypothesis

it would have to be assumed that the water entering the vacuolating cells has diffused through the side walls between the surrounding cells, the protoplasts of which have remained relatively impermeable.

The first possibility was explored by a microchemical examination of the cell walls. All the cells have cellulose in their walls, as indicated by the production of a blue colour on treatment with chloriodide of zinc, or a solution of iodine followed by 60 % sulphuric acid. At first this reaction was not evident, owing to the presence of the masking substance regularly found in young cell walls and removable by warming with Javelle water or with a dilute (3 %) solution of potassium hydroxide. No difference in the cellulose was discernible between the two lots of cells. The pectic layer of the cell wall in each case gave the reaction for pectic acid—it was insoluble in hot water, which would have dissolved any pectin present, and it dissolved completely when warmed with a 2 % solution of ammonia without previous treatment with acid, indicating absence of calcium pectate. A treatment with warm 3 % sodium hydroxide followed by copper oxide ammonia dissolved the walls completely, indicating the presence, in addition to the masking substance referred to above, of none of the ordinary cell wall materials but pectic acid and cellulose. The cellulose of young cells of this kind is well known to be easily extensible, and the calcium pectate, which would have hardened the cell walls, was absent from the walls of the non-vacuolating as well as the vacuolating cells.

Evidence against the second possibility—that of a higher osmotic pressure in the sap of the vacuolating cells, is to be seen in Pl. 1, fig. 3. It will be noticed that in preparing the tissues for sectioning, the enlarging cells have become partly plasmolysed. Plasmolysis of these particular cells could be prevented only with difficulty, while with the smaller cells near the veins, no such difficulty was encountered. In fact the smaller cells could be plasmolysed only after prolonged treatment with highly concentrated solutions. If the osmotic pressure of the vacuolating cells were sufficiently high to enable them to enlarge rapidly by extracting water from the surrounding small cells, one would expect the latter to plasmolyse more easily on treatment with a hypertonic solution.

All the findings are in agreement with the third hypothesis, namely that the enlarging cells vacuolate by virtue of a change in the permeability of their protoplasm leading to a rapid absorption of water, which reaches them through the side walls of the comparatively impermeable cells adjacent to the vein. The relative permeability of the various cells of the leaf is well demonstrated by the following experiment. Sections a few cells thick were cut from a young leaf at the stage when its lacuna-forming cells were just about to enlarge, but were still little if any larger than the rest of the mesophyll cells. At this stage all the mesophyll cells had small but unmistakable vacuoles. They were mounted in a concentrated solution of sucrose, and cells which had not been cut were observed microscopically. Within 2 min. plasmolysis had taken place in the cells about to form spaces. Similar shrinkage was observed in the other mesophyll cells and the cells of the palisade layer only after ½ hr. The epidermal cells, many of which were enlarging to form hairs, also indicated the permeability of their protoplasm by rapid plasmolysis.

The behaviour of the masking substance in the cell walls during the development of the leaf tissues is of interest. Tupper-Carey & Priestley (1923) noted that the cellulose walls of certain apical meristems failed to react with iodine and sulphuric acid, or with chloriodide of zinc. The meristem walls of a non-etiolated stem gave the typical reaction with iodine and sulphuric acid but with chloriodide of zinc they failed to respond without treatment. These authors suggested that the walls of young cells were composed of a complex of cellulose and pectic substance with protein and fat or, in the case of green stem meristem, with fat alone.

In the same year Hansteen-Cranner (1919) came to the conclusion that the wall in young cells is permeated by the plasma membrane of the cell, that is by a specialized outer layer of the cytoplasm which by its contained lipoids regulates the permeability of the cell.

In young leaves of *Ledum* with the veins differentiating, but as yet with no sign of air space formation, the cellulose of the mesophyll and epidermis stains lightly and that of developing wood cells stains deeply with iodine and sulphuric acid. With chloriodide of zinc, only the vascular bundles and a mass of vacuolated cells beneath the midrib become blue. After treatment with hot Javelle water or hot 3 % sodium hydroxide solution a good reaction is obtained with either reagent from all the tissues.

When the cells that produce the spaces have begun vacuolation, their walls become reactive with chloriodide of zinc. The same is true of the epidermal cells at the time when many of them are extending to produce hairs. The reaction in the vascular bundles also begins when their cells are vacuolating and elongating rapidly. In every case the acquirement of ability to vacuolate rapidly and to plasmolyse easily is accompanied by a change in the cell walls facilitating the cellulose reaction with chloriodide of zinc. These facts fit in well with the conclusion of Hansteen-Cranner that the cell walls are permeated by living protoplasm when this conclusion is combined with the further suggestion that the capacity for vacuolation is due to a change in the protoplasm which renders it more permeable. However, the present work does not support the further contention of Hansteen-Cranner that the protoplasm in the cell wall is a special layer, less permeable than the rest, for we must assume that water which is excluded from the small cells around the veins passes through the side walls of these cells to reach the rapidly vacuolating cells of the developing lacuna.

In the light of McPherson's theory as to the production of lysigenous air spaces, it is interesting to note that the increasing permeability and vacuolation of the enlarging cells, leading finally to a complete loss of their semi-permeable character and resulting collapse, is just the sequence of events that might be expected on the hypothesis that these cells lack food and are repairing their own proteins. At this time chlorophyll has begun to develop, but the leaf is growing rapidly and there is reason to believe that it is not yet in a condition to supply all its food requirements by means of photosynthesis. Mature leaves commonly contain quantities of starch but in no case has there been any evidence of starch formation in a young leaf of the kind we are considering. Under these conditions the cells farthest from the

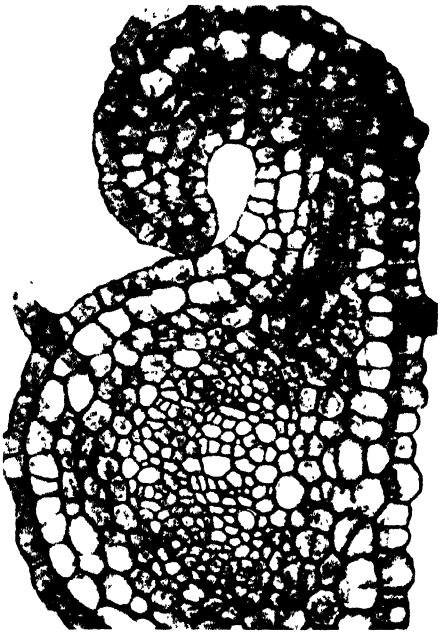

Fig. 1.

Fig. 3.

Fig. 2.

Fig. 4.

SIFTON—LYSIGENOUS AIR SPACES IN THE LEAF OF LABRADOR TEA

veins would have available only such food as passed by the actively growing, though slowly vacuolating cells between them and the source of supply. Thus a condition of starvation might well arise. The available evidence then, suggests that in this case as in that investigated by McPherson, the production of lysigenous air spaces may be due to an insufficiently rapid transport of good materials to the cells, and their consequent disintegration.

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EXPLANATION OF PLATE 1

Fig. 1. Transverse section of leaf near the outside of a bud collected 1 November. $\times 500$.

Fig. 2. Transverse section of mature leaf showing lysigenous air spaces. $\times 160$.

Fig. 3. Transverse section of leaf from opening bud collected 21 May. Beginning of air-space formation. $\times 500$.

Fig. 4. Transverse section of leaf from same buds as fig. 3. Further stage in development of air space. $\times 500$.

FORMATION AND DIVISION OF BINUCLEATE GIANT CELLS IN *MICRASTERIAS AMERICANA* (EHRENB.) RALFS.

By M. ROSENBERG

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(With 3 figures in the text)

In experiments on zygospore formation in desmids *Micrasterias americana* was mostly used, as it proved to respond very readily to changes in culture conditions. Various sets of conditions were tested with regard to their effect on the cells, light, temperature and the chemical constitution of the nutritive solution being the main variables. Full precautions were taken to ensure the purity of the distilled water and chemicals used, and the culture dishes were of Pyrex glass with very low alkali solubility.

It has been found by various workers that, if a sufficiently strong light source is provided, cultures of many species of algae can be made to grow well. In choosing the nutrient solutions other important factors such as temperature and light must be considered. As shown for *M. rotata* (results not published) the division rate is directly dependent upon the balance of factors, "the set of conditions".

One of the many sets of conditions used with the aim of inducing zygospore formation in *M. americana* in culture was darkness, low temperature (8° C.), nutritive solution Benecke 250 p.p.m., the constituents being:

NH_4NO_3	0·1 g.	K_2HPO_4	0·05 g.	Distilled water, 1000 ml.
$CaCl_2$	0·05 g.	$MgSO_4$	0·05 g.	

The effect, as had been anticipated, was a very low division rate. An examination of three parallel cultures in Benecke solution after a period of 10 days showed that a certain proportion of the cells were much larger than usual, showing an additional part between the two halves which normally join at the narrow isthmus, characteristic of all Placoderm desmids (Fig. 1). The proportion of giant to normal cells was 23 : 227; about 10 % of the cells subjected to conditions unfavourable to ordinary division developed into giant cells.

These giant cells were isolated and placed under conditions for rapid division to test their viability, and possibly throw some light on their origin. The new central part of these cells, usually barrel-shaped, shows a structure very different from the normal flat cells of *M. americana*, various lobes and branches protruding in diverse planes from the central part.

A number of series of cultures, starting with isolated giant cells, invariably gave the same result: inspection at any given time showed one and only one giant

cell—the "original"—and a number of perfectly normal cells of ordinary size and shape. The result of one out of many series was that six parallel cultures in Benecke solution, starting with one giant cell each, showed after 7 days one giant cell, and twenty-five, thirty, fourteen, twenty, fourteen, and twenty-two normal cells respectively. The series of camera lucida drawings (Fig. 2) shows a giant cell dividing, and gives—combined with other evidence—an explanation of the nature of this new type of cell. Divisions occur at both isthmuses simultaneously, and the formation of the new cell ends—the term "cell half" cannot rightly be applied in this case—occurs at the same rate at both ends. Clearly this method of division indicates the presence of two separate nuclei situated at the two isthmuses of the giant cell. These two nuclei can be seen at various stages of mitosis in the living cell, as the protoplasm

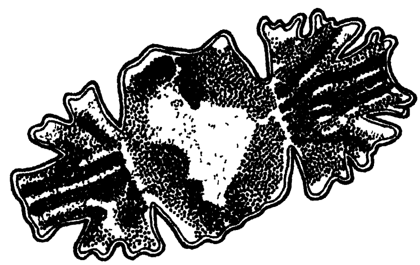

Fig. 1. *Micrasterias americana* giant cell, magnification × 350

is very transparent, the chromatophores not extending into the isthmus. While the new buds are developing and growing at both ends of the barrel-shaped central part of the giant cell, new halves are, again almost simultaneously, developing on the two halves which previously formed the flat ends of the giant cell. These buds, as well as those formed by the barrel-shaped part, develop into perfectly normal *M. americana* halves. The two normal cells so formed keep on dividing normally, like any other cell of the species resulting from undisturbed, normal division.

The result of every division of a giant cell is therefore one giant cell and two normal cells, due to the simultaneous division of the two separate nuclei within the giant cell. The membrane of the barrel-shaped central part remains unchanged in the cell, which continues to divide in the same way, as long as favourable conditions are provided.

Several series of experiments showed that giant cell formation takes place when conditions are unfavourable for division in general. In an old culture, for instance, where the solution had not been changed for 6 weeks—whereas it is usually changed every week for a similar bulk of cells—the proportion of giant to normal cells was 29 : 361. Experiments are in progress to ascertain in more detail the conditions necessary for giant cell formation.

The process that must take place at the formation of a giant cell is illustrated in Fig. 3. Nuclear division is completed normally, but plasmatic cleavage is prevented at an early stage. The process of swelling, which normally occurs at every division—the stage of bud formation—is, however, initiated. The result of this disturbed division process is however, a well balanced new system with two individual nuclei,

which in future divisions are perfectly co-ordinated, but separated by the whole length of the central part of the cell. Divisions occurred simultaneously at either end in over sixty cases observed.

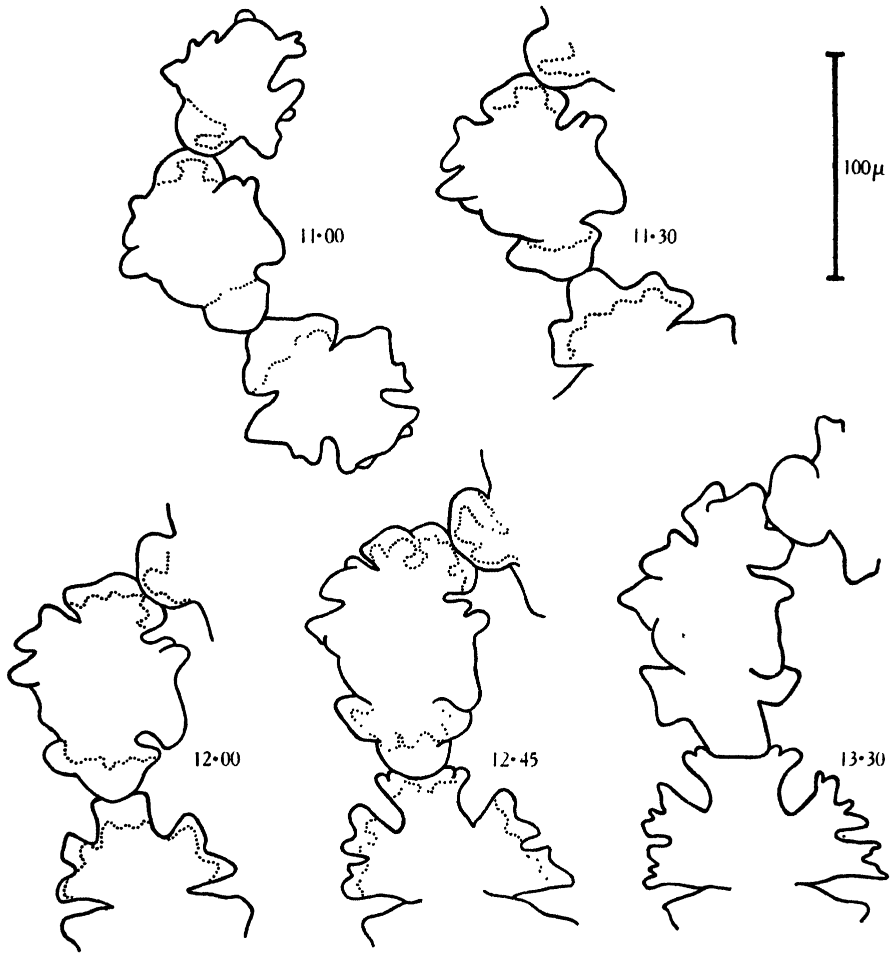

Fig. 2. *Micrasterias Americana*. Division of giant cell. Nuclear division was complete before the beginning of this series. Successive stages observed at intervals of 30 minutes or more as indicated.

Parallel experiments to those with *M. americana* were performed with *M. rotata* and *radiata*, the cultures being subject to the same sets of conditions, but in neither species could giant cell formation be observed.

A physiological explanation and understanding of the process of giant cell formation is not possible at the present stage, but it is clear that it is directly dependent on conditions in the environment, and can be induced at will; but these conditions themselves do not seem to be specific. We are dealing rather with a complex of factors tending to slow down the normal activities of the cell, inhibiting plasmatic cleavage but not mitosis. This giant cell formation is of considerable

theoretical interest, providing promising material for an experimental study of the causal relation between mitosis and plasmatic cleavage. It is a clear example of the essential independence of the two processes which are normally strictly coupled, but which are, as can be proved in some cases, due to different causes. It is possible in *M. americana* to follow the stages of mitosis in the living cell, and to subject the cell to changed conditions at any given stage. The division rate—under favourable conditions—is high, and the technique of culture not too difficult. The existing theories of cell cleavage (Gray, Just, Chambers, and others) have all been formulated and tested on animal cells, such as sea urchin and *Ascaris* eggs. In all these

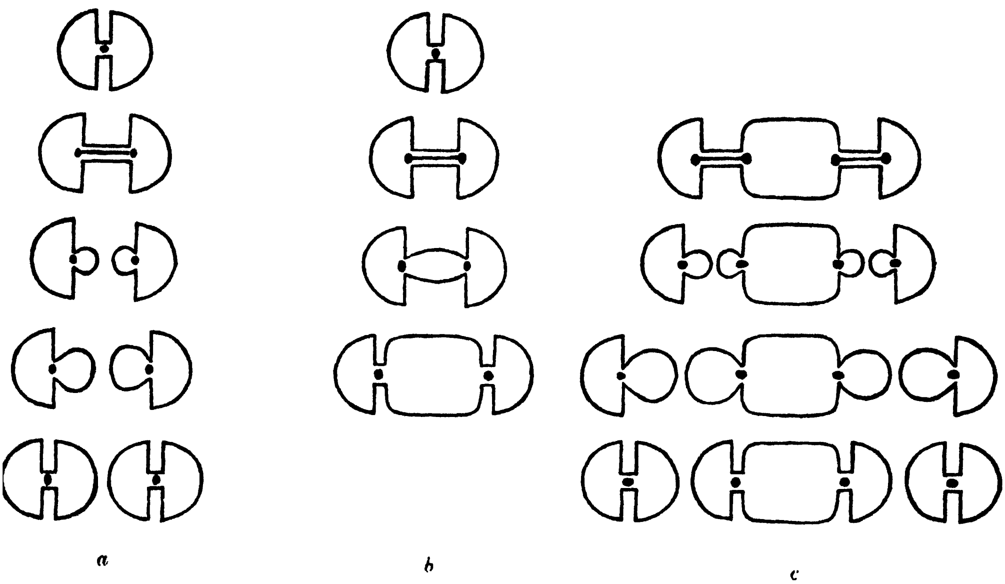

Fig. 3. Diagram showing (*a*) division of normal cell, (*b*) formation of giant cell, (*c*) division of giant cell.

cases, the presence of an aster plays a significant part in the theory. In a plant cell, such as *M. americana*, where there is no aster formed during mitosis, it can be hoped to find an explanation of cell cleavage of a more general nature, one applicable to the large number of cases where cell division occurs without aster formation.

Another aspect of the evidence for binucleate giant cells of the kind described is their influence on division rate. If under certain conditions the giant cells are formed, cell production following their appearance would be very much higher, two cells being produced at the same time, by one binucleate unit. This should lead to a rapid dominance of the species in a given habitat.

The occurrence of giant cells in the field has been recorded by many authors; it is, however, by no means frequent. In well over a hundred collections from localities in the English Lake District only twice have giant cells been observed. One case was that of *Staurastrum mucronatum* Ralfs, the other was *S. Arctiscon* (Ehr.) Lund. In both cases the collections were made during the winter months,

when, as a rule, divisions are very rare or do not occur at all. It is at this time that one would expect disturbed divisions resulting in giant cells, in analogy with the experimental evidence obtained to date.

Giant cell formation has been observed by various authors in some twelve species of different genera of placoderm desmids. Ducellier (1915), Laporte (1931), and Krieger (1933) have compiled the literature. Viret (1910) records a giant cell of *Micrasterias americana*, which was collected in July at an altitude of 2000 m. Although the presence of a nucleus in each of the two isthmuses of the abnormal cell has been recorded in several cases, in only one case (Czurda, p. 80), is there any mention of division or further development of the giant cells. According to observations here reported it may be assumed however, that these binucleate cells with additional central structures are potentially viable in all species, and may divide whenever conditions in the environment are favourable. It is intended to attempt a further analysis of the factors responsible for the formation and division of giant cells in *Micrasterias* and *Staurastrum*. In view of the binucleate nature of the giant cells it is of interest to point out a possible interpretation of observations by G. Huber-Pestalozzi (1927). In a paper on aplanospore formation in desmids he records a giant cell—with a barrel shaped central part—of *Cosmarium garrolense* Roy & Bisset, with the cell contents contracted in the centre, forming an "aplanospore", according to his terminology. Apparently only this one cell was observed, so that the possible presence of two nuclei at an earlier stage could not be confirmed, and Huber-Pestalozzi does not mention this possibility. It seems, however, very probable that two nuclei had been formed, which would allow a more plausible interpretation of the origin of the spore, which would thus be a zygospore, the result of an act of autogamy. Zygospores are as yet unknown in *C. garrolense*, so that a comparison with other figures is impossible. Two cases of "adelphogamy", the fusion of sister cells, have been described in the desmids. One has been described by Nieuwland (1909) for *C. bioculatum*, Breb. and is discussed in Kniep (1928). The other very convincing case is that of *Closterium Acerosum* (Schrank) Ehrenberg, for which Wenderoth (1935) records a continuous series of observations, starting with the formation of sister cells and ending with their fusion and the formation of a zygote. He has observed several sister cell pairs in this species up to the completion of the fusion process. The occurrence of conjugation between sister cells, at least in some species of desmids, lends support to the interpretation of Huber-Pestalozzi's observations. It seems that to the two observations of adelphogamy, *Cosmarium garrolense* might be added as a case of autogamy. An essential test for the validity of this interpretation is, of course, a study of the nuclear behaviour, and this will be attempted if *Micrasterias americana* provides comparable stages. Very little indeed is known of processes leading to zygospore formation in the desmids, and it may well be possible that autogamy in binucleate giant cells is one of the rarely observed sexual processes in this group.

Only after this paper had gone to press did Lefevre's paper come to my notice. He grew many desmids in clone cultures and reports giant cell formation in nine species, none of which, however, belongs to the genus *Micrasterias*. In *Cosmarium*,

cells with four nuclei and three intermediate sections were observed. The conditions under which these cells occurred are, however, not clearly stated; they appear to cause a low division rate. Lefevre also observed the division of these multinucleate cells as soon as they were transferred to "better" conditions, and the production of normal type cells at either end of the giant cell, which he calls double or multiple cell.

Summary

Binucleate giant cells were observed in *Micrasterias americana* and can be produced experimentally in about 10 % of the cells subjected to treatment. Division of giant cells was observed, resulting in two normal cells and one giant cell, which goes on dividing as long as conditions are favourable. The formation of giant cells is discussed and their possible connection with autogamy in desmids.

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SEASONAL VARIATIONS IN *STAURASTRUM PARADOXUM* MEYEN

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(With 2 figures in the text)

In the course of a study of the algal plankton of Swithland Reservoir, some 7 miles north of Leicester, interest was aroused by certain variations which were noticed in the desmid *Staurastrum paradoxum*, and appeared to be seasonal. This organism was a fairly constant constituent of the plankton during the summer months, but never became dominant and rarely exceeded 1 % of the total number of algal organisms present. *S. paradoxum* is well known in Britain as a common plankton alga. Three forms have been distinguished in Swithland Reservoir: (*a*) a triradiate form having three arms to each semicell (Fig. 2*a*, *b*), (*b*) a biradiate form having two arms to each semicell (Fig. 2*f*, *g*), and (*c*) an intermediate form having three arms on one semicell and two on the other (Fig. 2*e*). The triradiate form is the one which apparently is most usually found. However, West and Carter (1923) figure the biradiate form, and it is reported as having been found in Sutton Park in Warwickshire. So far as the present writer is aware, the intermediate form has not previously been reported. The only other species of *Staurastrum* observed in Swithland Reservoir was *S. gracile*. In some localities types intermediate between *S. gracile* and *S. paradoxum* occur, making identification of individual specimens difficult. Fortunately this was found not to be the case in Swithland Reservoir, and no serious difficulty was encountered in allocating specimens to one species or the other. The triradiate form of *S. paradoxum* is known in a number of varieties, viz. var. *longipes*, var. *cingulum*, var. *nodulosum*, var. *parvum*, and var. *evolutum*. In a few instances it was possible to place individuals in such varieties, but, in general, intergrading of the types occurred to such an extent that they could hardly be regarded as having varietal rank.

It is with the relationships of the triradiate, biradiate and intermediate types of *S. paradoxum* that this paper is mainly concerned.

During the year 1938–9 plankton samples were collected from the surface water of Swithland Reservoir at fortnightly intervals. Each sample was examined systematically, and of the first hundred specimens of *S. paradoxum* encountered each was allocated to its class, triradiate, biradiate or intermediate. In a few cases it was practicable to count only fifty individuals, on account of their rarity, and for the same reason it was not possible to obtain counts from October 1938 to April 1939 and from 31 July to 28 September 1939. The results are shown graphically in Fig. 1.

It may be seen from Fig. 1 that in the autumn of 1938 the biradiate form was clearly dominant; this was also the case in the spring of 1939. The proportion of

biradiate individuals fell rapidly during April and early May, and at the same time the number of intermediate organisms increased to 20%. Several examples were found where a biradiate individual was dividing to give rise to one biradiate and one intermediate individual (Fig. 2*d*). This rise in the number of intermediate organisms was closely followed by a rise in the number of triradiate organisms. It seems likely, therefore, that at this time of year the triradiate developed from the biradiate form, since, during this period, new semicells whether derived from bi- or triradiate parents, tended to be triradiate.

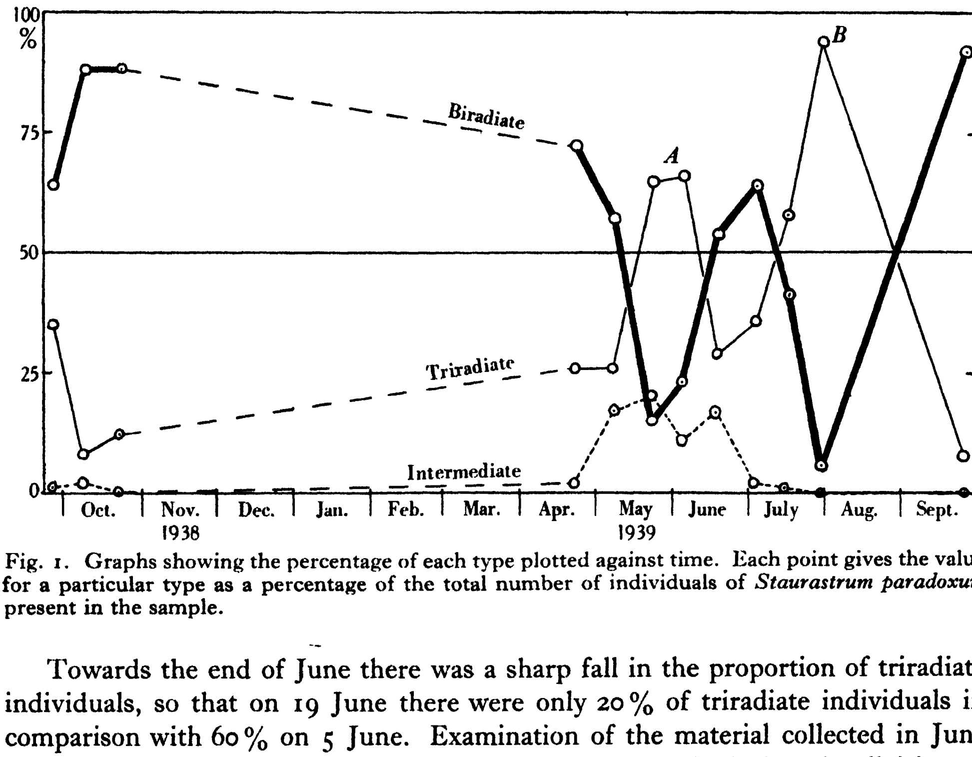

Fig. 1. Graphs showing the percentage of each type plotted against time. Each point gives the value for a particular type as a percentage of the total number of individuals of *Staurastrum paradoxum* present in the sample.

Towards the end of June there was a sharp fall in the proportion of triradiate individuals, so that on 19 June there were only 20% of triradiate individuals in comparison with 60% on 5 June. Examination of the material collected in June failed, however, to show the origin of biradiate semicells during the division of triradiate organisms, and, indeed, evidence of this occurrence was never found. This change back to dominance by the biradiate form appears to be due, not to the origin of biradiate from triradiate individuals, but to some other cause. The summer maximum of the triradiate form is in part to be accounted for by the origin of triradiate from biradiate forms, but this is not necessarily the sole factor involved. For instance, the maximum may be in part due to more active division of the triradiate form at this time of year, although no evidence on this point has been collected. The triradiate form was again dominant at the end of July, (Fig. 1 B). This second maximum appears to have been due to a different cause from the maximum in May (Fig. 1 A).

It is clear that the two forms of *S. paradoxum* are very similar and that they are not genetically distinct, since the one form may arise from the other. That being so,

one might expect that they would be affected more or less equally by changes in external conditions and would be equally liable to fungal attack. This, however, is not always so. An example of this was given by an attack on these organisms by a chytridiaceous parasite. The parasite appeared on the biradiate form on 4 July, when 6% were affected, but on this date no triradiate individuals were attacked. On 17 July, 84% of the biradiate form were parasitized, but only 2% of the triradiate were affected. A fortnight later the parasite seemed to have disappeared. Indeed, it was only in two samples, collected on 4 and 17 July, that the parasite was observed

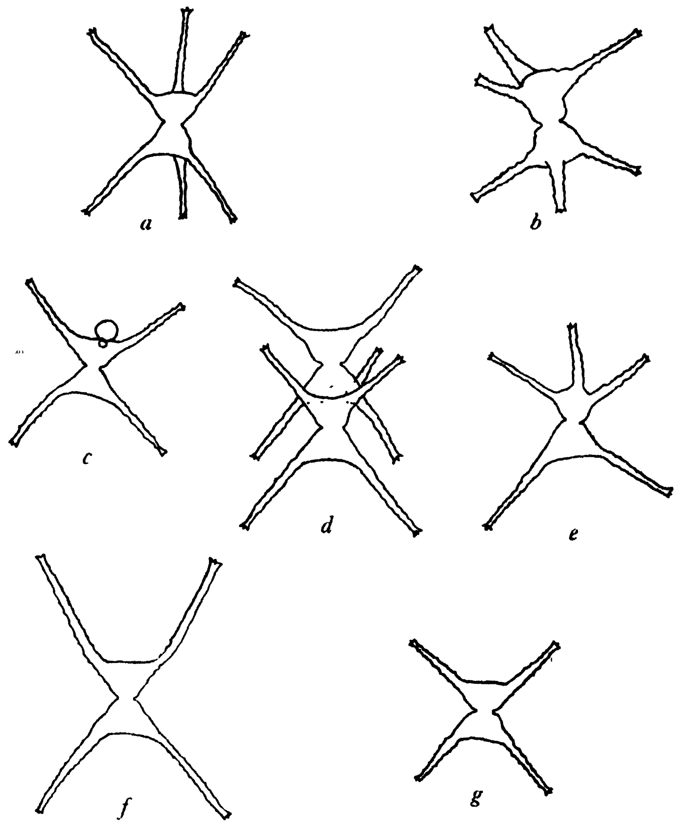

Fig. 2. *Staurastrum paradoxum* Meyen. (*a*) triradiate form, 19 June 1939, (*b*) triradiate form 17 July 1939, (*c*) biradiate form attacked by Chytridiaceous parasite, 17 July 1939, (*d*) triradiate semicell arising during the division of a biradiate individual, (*e*) intermediate form, 22 May 1939, (*f*) biradiate form, 19 June 1939, (*g*) biradiate form, 10 October 1938. *a, b, c, e, f, g* × 330; *d* × 310.

at all. It is unfortunate that intermediate forms were rare at this time as it would have been interesting to see to what extent they were affected. The parasite, which has not been identified, consists of a basal part immersed in the host, and a spherical zoosporangium produced externally (Fig. 2*c*). Often several parasites were found attached to one individual. In most instances the parasitized *Staurastrum* appeared very unhealthy and frequently the cell contents were disorganized.

One effect of this epidemic was to reverse the positions of the two forms. Whereas before the epidemic the biradiate form had been dominant, this position

was afterwards held by the triradiate form. Unfortunately soon after this the reservoir was heavily infested by *Anabaena* and this, together with the effect of the subsequent treatment with copper sulphate, made it impossible to obtain another count of the *Staurastrum* until 28 September when the biradiate form was again dominant, thus corresponding roughly to the condition of affairs in the autumn of the previous year.

As well as the changes in relative frequency of the two forms already discussed, changes were noticed in the form of the biradiate organisms. In general the arms tended to be longest in June, and to become shorter as winter approached, the length increasing again in the spring and early summer. The body of the organism did not appear to change in size to any considerable extent. The length of arm, for the purpose of measurement, was taken as the distance from the tip of the arm to the isthmus. The extremes of length observed were on 19 June 1939 (Fig. 2*f*), when the mean length was 57μ (the extreme values being 48 and 69μ), and on 10 October 1938 (Fig. 2*g*), when the mean was 36μ (with 28 and 40μ as the extremes). In each instance the mean value is the average of lengths of the arms of twenty randomly selected individuals. Since each individual has four arms, each result is the average of eighty measurements. The significance of the difference between the means was investigated statistically using "Student's" method (Fisher, 1928, p. 107). By this method it was found that the value of P was considerably less than 0·01 indicating that the difference of the two means is fully significant.

A similar variation in the lengths of the arms of the triradiate organisms was observed but was not examined in detail.

Summary

Staurastrum paradoxum occurs in Swithland Reservoir in three forms, (i) biradiate, having two arms on each semicell, (ii) triradiate, having three arms on each semicell, and (iii) intermediate, having two arms on one semicell and three on the other.

The biradiate form predominates in the autumn, winter and spring. The triradiate form appears to be a midsummer form. Evidence of the origin of the triradiate from the biradiate type is given.

The proportions of the three forms may be affected by causes such as fungal attack on one form.

The length of the arms of the biradiate form varies in a seasonal manner, the length being greatest in June and shortest in October.

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REVIEWS

Recherches expérimentales sur le Polymorphisme et la Tératologie des Desmidiées. Encyclopédie Biologique, XIX. By M. LEFEVRE. $6\frac{1}{2} \times 10$ in. Pp. 42 with 7 plates, 205 text-figures. Paris: Paul Lechevalier, 1939.

In recent years, since culture methods for algae have been developed and improved, experimental work on desmids has been carried out by various workers. Czurda summarized the work done up to 1937 in the *Handb. d. Pflanzenanatomie*, **6**, *Conjugatae*, and showed that some of the fundamental problems, such as variability and morphological changes, could be successfully studied experimentally. A causal relationship, or even correlation, between physiological and morphological phenomena has never yet been established; it must, however, remain the ultimate aim of this type of work.

Lefevre, in his book, gives very many interesting facts on variability and morphological changes but attempts no physiological explanation except in very general terms. He reports on experimental work carried out, together with field collections, during the last ten years, and interprets the findings of various authors in the light of his evidence from culture work.

Over thirty species from about ten genera have been grown in liquid or on solid media. All the cultures are clone cultures, but not bacteria free, and they start with one cell only, so that the material is genetically homogeneous, an essential character for this type of work. Unfortunately no mention is made in this paper of the composition of the media, only total concentrations occasionally being given. For any further analysis of the results it is essential that the conditions of culture be described quantitatively and in detail. None of the factors, light, temperature, or medium, can be ascertained from the text with any certainty, and this, of course, makes comparison with other work difficult. As far as the division rate, to which the author refers on various occasions, is concerned, it has been shown by other workers that it is an immediate result of the physical and chemical conditions of the environment, and directly depends on them. Division rates as quoted by the author are definitely low, which is most probably due to his particular set of conditions and could therefore be speeded up considerably. It seems very doubtful whether, in unicellular algae, a rhythm of division can be maintained after a change of conditions has taken place. The author suggests this on p. 24 as an explanation of a certain low division rate; all evidence elsewhere, however, seems to show a direct relation between a change in conditions and a change in the division rate, although this relation may be very complex.

Another point which makes a full comprehension of the results difficult is the fact that no figures or proportions are given for the aberrant cells obtained in culture. The different types of cells are very clearly illustrated by outline drawings and good photomicrographs, though it is to be regretted that no indication of the magnification is given.

In classifying the different cell types obtained the author distinguishes between abnormalities, or regular symmetrical changes, and monstrosities, or irregular unsymmetrical changes. He speaks of abnormalities where many or all cells in a culture are affected by a change of conditions, and of monstrosities where only one or a few cells show a reaction, and in this latter case the reaction appears always to be irregular. Although the causes are not understood in either group there seems to be some justification for a classification of the effects in this way, though the differences may be of a quantitative nature only.

The nucleus and its division have not been studied by the author. His observations begin just before the stage of bud formation, and are concerned with the effect of conditions on this susceptible phase of cell formation. In nine species the formation of "double cells" with two nuclei was observed in cultures with low division rates. This seems to be the first time—apart from occasional observations by Oudracek—that double cells have been produced in cultures. They have frequently been found in collections, but their formation is not yet clearly understood.

It is pointed out that the frequency with which abnormalities occur varies very much with different species, but it should be noted here that the conditions were restricted; unless

work is carried out under strictly controlled conditions, covering a wide range of possible combinations, such general statements cannot but be misleading. These observations, combined with field work, are, however, of some significance as indications of the kind of variations which may occur. These possible variations are very manifold, relating to the size and shape of the cells, and their ornamentation, such as spines and delicate structure of the membrane. In general one can say that solid media, or high concentrations of liquid media, e.g. 0·300 g. per litre, tend to give small cells with little or no complication of shape, the spines and points usually being entirely absent. The differences are sometimes so great that they might be considered as generic if it were not for the fact of the common origin of the changed cells from one individual.

Perhaps the most interesting point in the paper is the evidence the author has obtained of the reversibility of some morphological changes in either of his two groups. He shows that two, or even one, cell division under changed conditions can restore the original cell type. A striking case is *Arthrodesmus convergens*, which loses its spines and changes the cell shape entirely in a concentrated medium (0·300 g. per litre). Transferred to a concentration of 0·115 g. per litre, the first daughter-cell generation develops spines and the typical "normal" cell shape. Another experiment should be mentioned: typical *Staurastrum furcigerum* cells, showing six processes on every half cell, gave nothing but cells with nine processes under certain, unfortunately unspecified, culture conditions. When transferred to twice distilled water the number of processes was immediately reduced to six and remained at that. The form with nine processes agreed in every detail with the variety *Eustephana* (Ehrbg.) Nordst described from various localities.

The author concludes from his results that most desmids are polymorphous and that culture work must be carried out in conjunction with field collections in order to obtain a better understanding of the systematics and the range of species in this group.

It may be said, however, that although culture work of the kind presented in this paper augments our knowledge of the possible scope of morphological reactions of the cells, and allows us to trace the origin of successive cell generations, we do not get much further towards an understanding of the causes or of the nature of these changes. The bearing of this kind of work on systematics and morphological problems is obvious, but it seems that we need a more physiological approach if we are to come to a better understanding of the fascinating phenomena of modifications of genetically homogeneous material. The desmids seem in many ways an ideal group for future work on these lines.

M. ROSENBERG

Introduction to the Botany of Field Crops. By J. M. HECTOR, Professor of Agricultural Botany, University of Pretoria, 10 × 7 in. Vol. I. Cereals: pp. 478+xxxiv. Vol. II. Non-cereals: pp. 648+xxxiii. Plates and text-figures: 448. Johannesburg, South Africa: Central News Agency Ltd. Price £3. 10*s*. net per set.

The present book constitutes Vol. XVI of the "South African Agricultural Series". This fact might lead us to expect either that the author would deal exclusively with South African field crops, or else that he would pay especial attention to crops of particular interest to the farmers of South Africa. This, however, was obviously not his intention, and even after reading the book it would be difficult, if not impossible, to say which crops dealt with are peculiar to or important in South Africa.

The treatment, therefore, is intended to be quite general, but the author in his preface explains that "the term 'field crops' is here used in a particular sense: inclusively to incorporate all plants cultivated in the 'field', i.e. on arable land; exclusively to omit the plants of the garden, the orchard, the grove and the plantation. The distinction, however, is not absolute, since all 'cultivated' grasses are omitted, and certain plants, such as cotton—regarded in some areas as a 'plantation crop'—are included". Moreover, the treatment is not intended to be exhaustive. The author further states that "physiological aspects are dealt with only incidentally", while "one aspect...has been omitted—the viewpoint now generally known as 'crop ecology'". The reasons given appear to be satisfactory and adequate.

The book, although it represents only a single volume of the series to which it belongs, actually appears as two volumes. The first volume, entitled "Cereals", includes gramineous crops only, including the sugar canes, but, as intended, excluding "cultivated" grasses. The contents of the second volume, entitled "Non-cereals", are more varied. In this volume each of the thirteen Natural Orders, *Liliaceae, Moraceae, Polygonaceae, Chenopodiaceae, Cruciferae, Leguminosae, Linaceae, Malvaceae, Umbelliferae, Convolvulaceae, Solanaceae, Cucurbitaceae* and *Compositae*, in the order given, is allowed a separate chapter. The length of the different chapters varies greatly, however, as well as the number of pages given to each particular crop. Thus, in the first volume, which is entirely devoted to the *Gramineae*, wheat species take up about 25 % of the space, maize about 18 %, and oats about 15 %, and then, in decreasing order, the sugar canes, barley, rice, sorghums, rye and millet. In the second volume, more than half of the total space is taken up by the three Natural Orders, *Leguminosae, Malvaceae* and *Solonaceae*.

No absolute uniformity of design is followed in dealing with each crop. Since the work is very largely a compilation, using information from an extremely wide variety of sources, no absolute equality of treatment was possible, nor was it desirable, and on the whole it would seem that the length of each chapter, or the amount of space devoted to each particular crop has depended upon the amount of pertinent and relevant information (subject to condensation) available in the literature. In fact, the book might well have been entitled "Introduction to the Literature of the Botany of Field Crops", because, in the first instance it gives a student a very good idea of how to approach the literature of some particular aspect of Field Crop Botany in which he may be interested, while at the same time the bibliography at the end of each chapter leads him directly to that literature. This is very largely the author's intention, because he definitely states that "the book has not been written merely as a 'text book' in the accepted meaning of the term...it is an introduction to a study which is rapidly expanding ...an 'introduction' to its literature, comprehensive perhaps, but in no sense exhaustive; a beginning, in brief, and not an end".

The reader only tends sometimes to lose sight of this viewpoint where the author does, in fact, seem to deal with some aspect of his subject in very considerable detail. This, however, is not contrary to the general design because to an extent the book can well be used as a text book. From the reviewer's point of view, the work has a very special value in that it brings together in a relatively small compass an amazing amount of information upon aspects of botany with which he may not be immediately and directly concerned, but which are yet well within the scope of his general interest.

The author gives special attention to morphology, embryonic development, root and stem anatomy, floral development, anthesis, pollination, fertilization, cyto-genetics, and the development and structure of the fruit and seed. He is also obviously very interested in the contact of the plant with its environment, as shown by the attention given to root systems where details are available, but he advisedly withholds from dealing extensively with physiological problems as such.

It is not to be expected that a work of this magnitude should be absolutely faultless, or that all typographical errors should escape detection. Misprints, however, are by no means common and are usually obvious, but occasionally we find the wrong word used, a wrong chromosome number given, or a name wrongly spelt, and these may momentarily cause some slight confusion in the mind of the reader.

Such confusion is apt to arise particularly from the varying use of the symbols "x" and "n" (and also "X" on p. 811) in connexion with cytological and cytogenetical work. Thus, in two different places (Tables IV and VI) we find the hexaploid represented by "$6x$" and by "$6n$". Unfortunately, such instances could be considerably multiplied.

A rather different source of confusion is illustrated by the use of, say, "14/28" on pp. 38 and 39 to denote respectively the gametic and somatic chromosome numbers of a species, and the use of the same notation, "14/28" on pp. 49 and 50 to show that the somatic chromosome number is either 14 or 28.

The bibliography at the end of each chapter forms a valuable guide to the literature up to the year 1936, while at the end of each volume we find an "Author Index", a "Plant Index" and a "General Index". The compilation of these lists and indices alone would be quite an achievement, but when we realize that all this literature has been perused in the preparation of the book, the magnitude of the work is at once evident. Sometimes, however, the pursuit

of a reference given in the text fails to lead us to the actual source of the information. To quote a single instance, we find "Rimpau's hybrids (1891)" referred to on p. 225, but the direct reference is not given. Such instances are not common and obviously deserve sympathy rather than criticism.

Statements that require confirmation, and sometimes revision, also occur rarely. They are usually "slips of the pen", or the result of too intense condensation (e.g. on p. 900). This is the probable reason why on reading p. 776 (referring to Williams, 1931) we get the impression that (1) strain-building, (2) brother X sister mating and (3) diallel crossing, are parallel or alternative methods used in the improvement of red clover. Actually these refer to alternative uses to which F_1 material may be put in breeding for the production of improved strains. "Strain building" in the sense there used by Williams referred to a method by means of which promising material could be rapidly increased for practical use without resort to the more refined process that may involve either brother X sister mating and/or diallel crossing.

The author has been fortunate in being able to prepare or secure a wealth of illustrations and tables, but a few of those borrowed are not quite fully explained. Nomenclature purists might also object to the lack of uniformity in writing the names of genera and species, but the general botanist can afford to be tolerant in this connexion.

In conclusion, having mentioned some points that deserve further attention when a new edition is being prepared, we would emphasize the intrinsic value of the work as a whole. Not only does it summarize the work already done on the botany of a very wide variety of field crops, but by implication it also suggests lines of research on crop species that have hitherto received scant attention.

T. J. JENKIN

Das Holz als Rohstoff: seine Entstehung, stoffliche Beschaffenheit und chemische Verwertung. By DR REINHARD TRENDELENBURG. $6\frac{1}{2} \times 9\frac{3}{4}$ in. Pp. 435, with 108 text-figures. München and Berlin: J. F. Lehmanns. 1939. 14.50 gold marks.

The importance of wood as a constructional material has prompted many detailed studies of the anatomical structure, and its relation to physical and mechanical properties; these are described in various text-books that have appeared during the present century. New methods of intensive utilization, which will no doubt play an important part in the use of wood in the near future, demand a fuller understanding of the origin, composition and structure of the material than is contained in any of these books, and Dr Trendelenburg's account, written from a viewpoint between that of the plant physiologist who is also a forester, and the technologist, furnishes an up-to-date survey which admirably supplements the standard works. Two short introductory sections and one final section, intended primarily for German readers, give a general description of the distribution, composition and working of the German forests, and brief notes on the use of wood in the production of paper pulp, cellulose, sugar, charcoal, wood-gas, wood-wool and fibre-board.

The third section discusses variations in the sizes of the cells and relative proportions of the tissues, and quotes the standards of size classification recommended by the International Association of Wood Anatomists. In this section reference is also made to work on the principal chemical constituents of the cell wall and their probable influence on the properties of wood, and to current conceptions of the micellar structure. Section four summarizes information on the growth ring and the principal factors influencing diameter growth. There is an excellent account of sapwood and heartwood which includes the results of very recent investigations. Compared with other text-books, the amount of space devoted in the fifth and sixth sections to considerations of moisture content and specific gravity may at first sight appear to be disproportionately large and rather mathematical in parts. A closer examination will show, however, that such a comprehensive account is essential to a complete understanding of the material, and much of it is of real importance to the plant physiologist.

To English botanists the book will commend itself because of the introduction it affords to over 600 papers, more than 460 of which are in German, and because of the detailed index which runs to forty-five pages and makes the contents of the volume readily accessible.

S. H. CLARKE

The Economic History of the Kiowa Indians as it relates to the History of the Tribe. By P. A. VESTAL and R. E. SCHULTES. 11×7½ in. Pp. 110 with 4 plates. Botanical Museum, Cambridge, Mass. 1939.

It is a sign of activity and awareness that botanical science should at the present time be reaching out into contacts with many allied sciences, not least among them those directly concerned with human sociology and human history. The study of the utilization of plants and plant products by primitive races has attracted much desultory attention in the past, but such studies have often suffered from the serious difficulty of getting an adequately high standard on both the botanical and ethnological sides of the enquiry. The material has seldom led to results of general usefulness or of a striking character.

The publication under review is an instance, however, where thorough and careful botanical identification and exact citation are apparently linked to thorough ethnological treatment, and the problem, that of the economic botany of the Kiowa Indians, was one capable of yielding some interesting general conclusions.

The Kiowa Indians are a tribe of the western plains of North America, and their history, over the last two hundred years or so, is known with fair exactitude. The ancestors of the present Kiowa before 1700 lived in Western Montana, but about that year they migrated southwards across the Yellowstone River, where they settled north-east of the Black Hills and there acquired horses for the first time, an event which revolutionized their economy. From about 1780 they were forced by hostile tribes to migrate slowly south. In the middle of the nineteenth century they came into conflict with the United States troops and in 1879 they were finally forced to settle in Oklahoma where the reservation still exists in which these present enquiries were made.

All the plants now growing in the Oklahoma region and spoken of by the oldest Kiowa Indians as used formerly or still used by the tribe, were identified by the authors and their uses recorded. The data were then analysed in terms of two factors, the uses known to be made of these plants by other tribes, and the geographical distribution of the plants. The Kiowa had migrated so far that they had passed into a region of substantially different flora from their ancient home, and very interesting results emerged from a grouping of the utilized species according to their range.

Those species which extended from Oklahoma to Montana might have been employed before and during the migrations. Within this class are many plants still of fundamental economic importance to the tribe: the use of others has almost ceased. It is a striking fact that the species which extend less far but nevertheless reach the Black Hills region, yielded to the Kiowa plants particularly related to travel, war, to the horse and the buffalo, and it is noteworthy that the uses made by the Kiowa of these plants are strongly localized to this tribe. They include medicines, tanning agents, temporary bedding and so forth.

The third group, which includes species extending to Wyoming, Western Nebraska and Colorado, reflects just the same nomadic life as the preceding group, and no doubt this group includes many species brought into use at the time when the Kiowa and Comanche tribes jointly dominated this part of the plains. The fourth group includes species which could hardly have been met with before the Kiowa reached the Oklahoma reservation, and takes in species introduced by white men and now established in America. This group, in contrast with the preceding groups, includes very few medicines and little which relates to the buffalo.

The last group consists of a few species with which the Kiowa became cognizant during their raids into Texas, Mexico and the south-west. It includes the Mesquite, the Mescal Bean (*Sophora secundiflora*), the Bottle Gourd (*Lagenaria siceraria*) and the Peyote (*Lophophora Williamsii*). The last three play an important part in the widespread religious peyote-ceremony which was introduced also from the south between 1880 and 1885.

So far as can be judged, the borrowing of uses from other tribes has played little part in the acquisition of the economic botany of the tribe... and this maintains the fortunate clarity with which their knowledge of useful plants can be correlated with the three major phases of the recent history of the Kiowa.

The data in such a study as this are complex and the authors handle them with appropriate caution: the broad outlines emerge quite clearly nevertheless, and clearly demonstrate the

value of this kind of co-operative work. Thus far the gain has been mainly that to ethnology, but by developing such studies as these, botanical science may possibly in return learn something of the important question of the extent to which primitive man has himself contributed to the modification or determination of plant distribution. H. GODWIN

Protein Metabolism in the Plant. By A. C. CHIBNALL. 9 × 6 in. Pp. xiii+306 with 9 plates and 21 figs. in the text. New Haven: Yale University Press; London: Humphrey Milford. 1939. Price 18*s*.

Prof. Chibnall has joined the distinguished company of those who have delivered the Mrs Hepsa Ely Silliman Memorial Lectures at Yale University. It is a provision of the benefaction that the matter of such lectures shall afterwards be published in book form. Prof. Chibnall has taken as his subject two aspects of the higher plant's nitrogen metabolism viz. the protein metabolism of seedlings and the corresponding activities in leaves. This choice has enforced a division of the matter into two very dissimilar parts. The relation of seedling proteins to co-existing amides and amino-acids, being a classic field of plant-nitrogen studies, has been summarized many times already. It may be doubted, however, whether the early theories and controversies have ever been made so fully and conveniently accessible in English before.

It is, nevertheless, to the later chapters dealing with leaf metabolism that one turns with the greatest interest; since it is to this field that Prof. Chibnall's own considerable contributions belong. On p. 47 the author pokes gentle fun at Schultze for declining to discuss a theory for lack of evidence and taking 2000 words to do it; but on p. 241 he winds up an original discussion of his own by calling it "admittedly speculative"; it has then run, on a rough estimate, to 9000 words. The same epithet is applied by the author to the second section of the book as a whole, and one does indeed feel that some parts of it would perhaps be more properly at home in a laboratory notebook than in a published monograph.

The poet accused of telling lies replied that at least they were very beautiful lies, and there could be no answer to the defence that this is very fascinating speculation. To describe it fully is impossible and to select details somewhat unfair, but the following quotation from the last page but one may give grounds for thought not only perhaps to its readers: "If it be assumed that a protein cycle were operating in these" (cut tobacco) "leaves at the same speed as in barley, and that the normal rate of protein degradation had been maintained after detachment from the plant, then this loss would suggest that the rate of protein synthesis had by some means been decreased by one-seventh." The context leads one to suppose that "by one-seventh" may be a misprint for "to one-seventh", but it also increases the number of evident hazards on which the calculation hangs: it is, for example, explicitly stated that different species of leaves differ very much in their rates of proteolysis. One cannot help feeling that little is to be gained by pursuing such pseudo-numerical arguments as this.

Having entered this caveat we come to the main point viz. that a great deal of recent work, some of it not very easy to come at in this country, is here summarized with obvious authority. Particularly interesting is the account of the ether-water extraction method developed by Prof. Chibnall himself, including the investigation of leaf proteins and the co-ordination of macrochemical and cytological data which it has made possible. At the present time, when a definitive presentation of the subject is scarcely possible, these pages must lay its students under a heavy debt even though one must often borrow the beginning of the author's last sentence: "On this important question, however, I would prefer to reserve judgement...." W. O. JAMES

ENDOCOENOBIUM EUDORINAE GEN. ET SP.NOV., A CHYTRIDIACEOUS FUNGUS PARASITIZING *EUDORINA ELEGANS* EHRENB.

BY C. T. INGOLD
Department of Botany, University College, Leicester

(With Plate 2 and 4 figures in the text)

IN the course of an examination of the plankton of Swithland Reservoir, seven miles north-west of Leicester, it was noticed, during the autumn of 1939, that *Eudorina elegans* was heavily infected by a chytridiaceous fungus. In searching through the literature I failed to find any reference to this fungus. I therefore consulted Dr F. K. Sparrow, who has done so much in recent years to further our knowledge of the Chytridiales, and he tells me that the fungus is new and departs so much from other species that it deserves to be placed in a new genus in the sub-family Polyphageae of the Rhizidiaceae, one of the most interesting families of the Chytridiales.

I would suggest the following diagnosis:

Endocoenobium gen.nov.

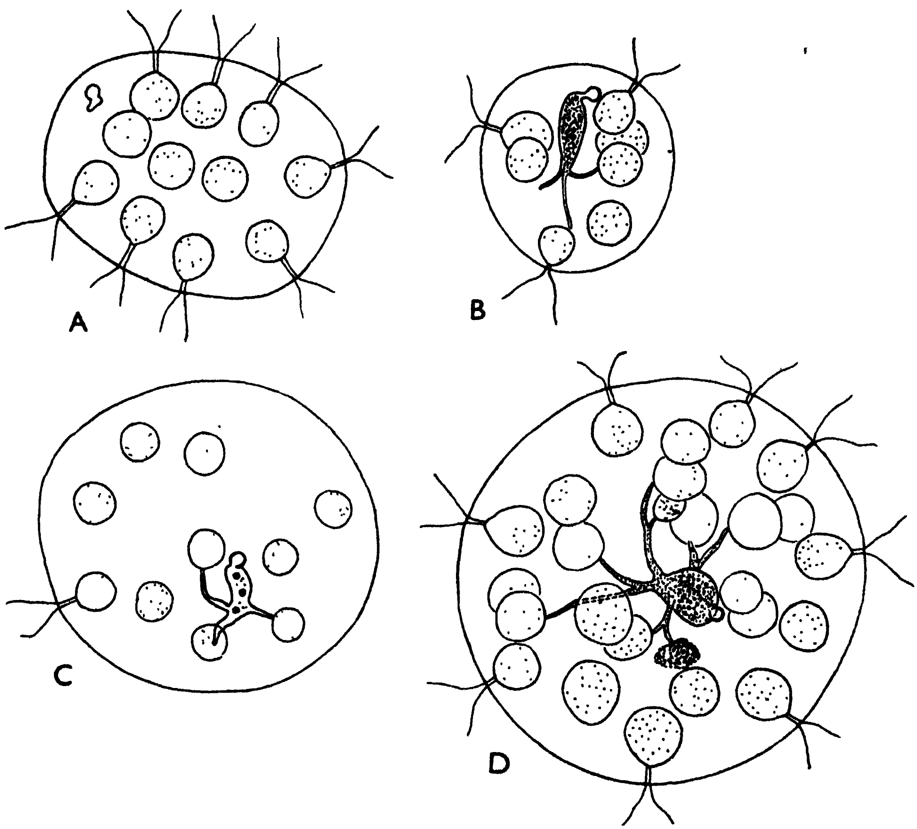
The thallus is microscopic, living within the coenobium of members of the Volvocales. From the sac-like thallus rhizoidal outgrowths, branched or unbranched, make contact with the host cells. The thallus is *not* produced by the direct enlargement of the encysted zoospore, but by the enlargement of an outgrowth from it. The encysted zoospore persists on the surface of the host coenobium. The zoo-sporangium is elongated, and cut off from the thallus by a cross wall. Dehiscence is by an apical tear. The zoospores are uni-ciliate. The thick-walled resting spores are produced following a sexual process. In this process two thalli, which have developed in the same coenobium, fuse, and from the fusion-cell a warted zygospore is budded off.

The genus is represented by a single species, *Endocoenobium Eudorinae* sp.nov. which is discussed in detail below. The description of the fungus is based entirely on observations made on living material, and no cytological examination has been undertaken.

ASEXUAL CYCLE

The uni-ciliate zoospore (Fig. 2H), which is of the normal chytridiaceous type, is nearly spherical and 5μ in diameter. It is naked and has a single highly refractive spot, probably composed of oil, close to its periphery. Near this spot the single long cilium is inserted. The zoospore comes to rest on the surface of a motile *Eudorina* coenobium and surrounds itself with a wall. From the encysted zoospore a short process grows out which becomes firmly attached to the wall of the coenobium

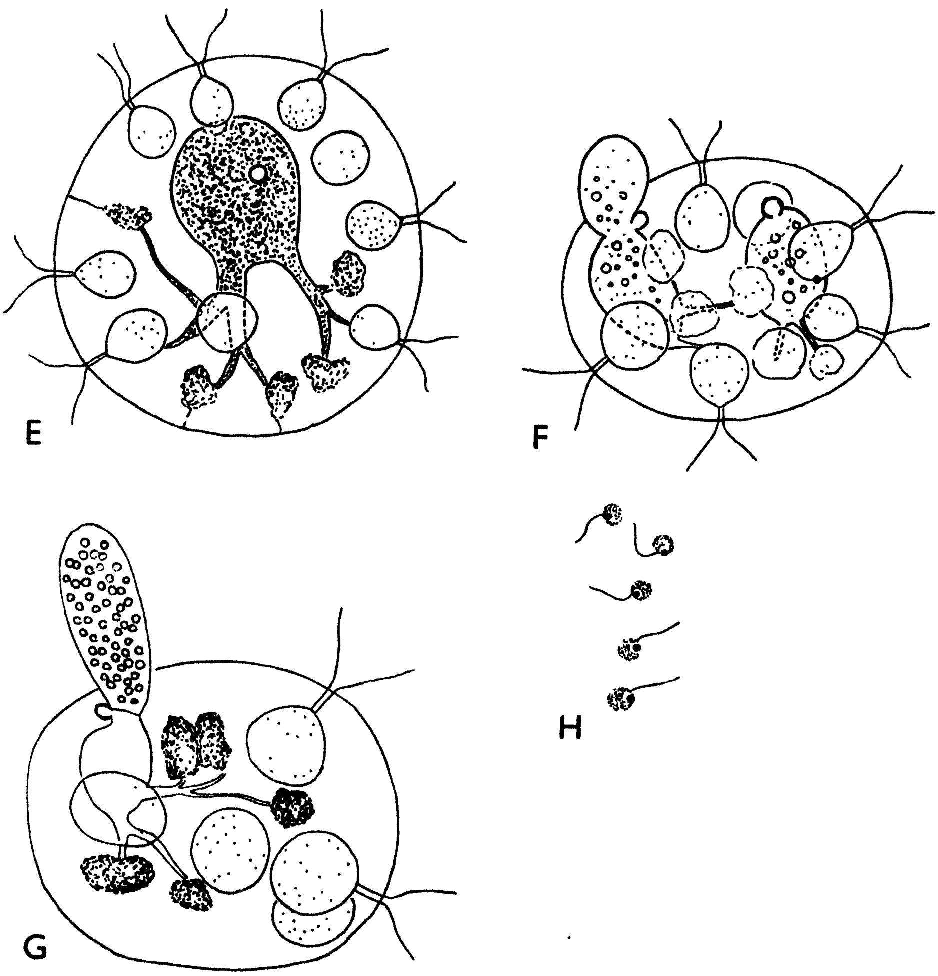
(Text-fig. 1A). This represents an appressorium comparable with that described by Sparrow (1936) in the development of *Scherffeliomyces parasitans*. From the appressorium a tube is formed, which enlarges within the coenobium into the vegetative body of the fungus. There develop from this thallus mycelium-like outgrowths which grow towards, and make contact with, the cells of the coenobium (Text-fig. 1B, C, D). Sometimes the rhizoidal outgrowths are simple, but often some of them are branched. A single thallus may tap up to a dozen cells of a *Eudorina*



Text-fig. 1. *Endocoenobium Eudorinae*. A, early stage in infection; the zoospore, having come to rest on *Eudorina*, has given rise to an appressorium on the surface of the coenobium. B, C and D, development of the thallus; the zoospore-knob is in all cases on the surface, while the thallus is inside the coenobium. In A and C only the front cells of the *Eudorina* coenobium are shown. Drawn with camera lucida from living material. × 473.

colony. The host cells which are attacked soon show signs of disease, and their contents aggregate, change colour and shrink. Provided, however, that at least one or two cells of the coenobium remain unaffected, the coenobium still retains its motility. Concurrently with the development of a diseased appearance in the host cells, the parasite enlarges within the coenobium (Text-fig. 2E). The contents at this stage are dense and oil drops can usually be recognized. Finally a zoosporangium is produced which projects from the parasitized coenobium (Text-fig. 2F, G

and Pl. 2, no. 1). The zoosporangium always grows out at the side of the old encysted zoospore which remains as a little knob on the surface of the *Eudorina* coenobium. In the formation of the zoosporangium the entire protoplasmic contents pass into it, and it is finally cut off from the hyaline thallus by a cross wall. The sporangium



Text-fig. 2. *Endocoenobium Eudorinae.* E, fully grown thallus; the zoospore-knob, directly above the main body of the thallus, is actually at a higher focus on the surface of the coenobium. F, a *Eudorina* infected with two asexual thalli; the one on the left is beginning to grow out to produce a zoosporangium. G, a thallus with a mature zoosporangium; the zoospore-knob is immediately to the left of the sporangium; the little circles in the zoosporangium are oil drops, and each indicates the position of a zoospore. H, zoospores. Drawn with camera lucida from living material. × 578.

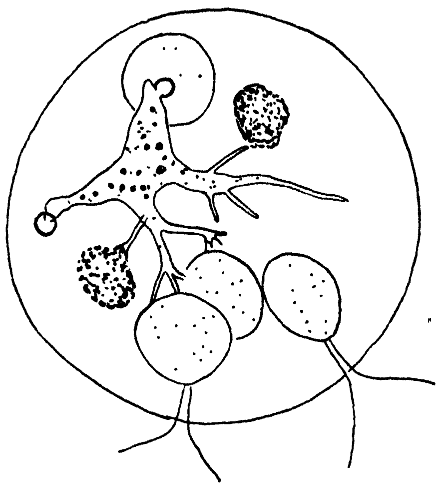
is about $45\,\mu$ long by $18\,\mu$ broad and within it between fifty and a hundred zoospores are matured. It is a very striking sight to see a *Eudorina* colony swimming about with this relatively large zoosporangium projecting from it. I have seen six mature zoosporangia, and in two cases I have watched dehiscence and the escape of the zoospores. When it is ripe the sporangium opens by an apical tear, there is no lid,

and the zoospores swim out singly. The rigidity of the intact sporangium is due to its turgidity, since when the zoospores escape the old sporangium wall is left as a thin shrivelled envelope.

FORMATION OF THE RESTING SPORES (ZYGOSPORES)

In plankton catches made in Swithland Reservoir at the end of September 1939 only the asexual stage of *Endocoenobium* was observed. In material collected throughout October both asexual and sexual stages occurred, but in the later collections the sexual stages predominated.

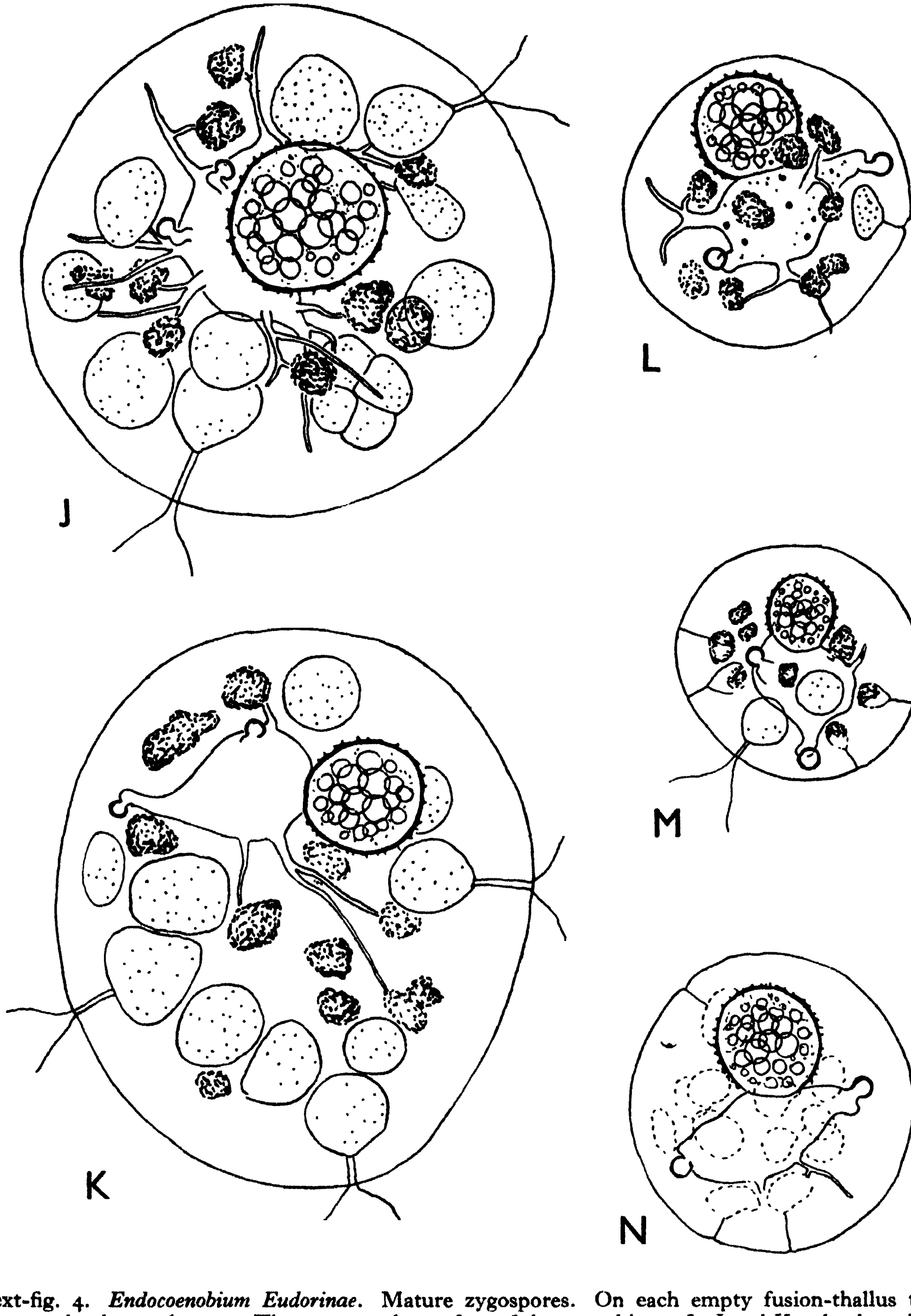
Sexual fusion occurs when a *Eudorina* is attacked by two zoospores, and in consequence two separate thalli develop within the same coenobium. The presence of two thalli in the same *Eudorina* does not always lead to fusion. Each may develop



Text-fig. 3. *Endocoenobium Eudorinae*. Sexual fusion. Two thalli have united to form a fusion-thallus. The black dots are *not* nuclei. The zoospore knobs are on the surface of the *Eudorina* coenobium. The fusion-thallus is within the coenobium. Drawn with camera lucida from living material. × 650.

asexually (Text fig. 2F). However, where a resting spore is formed two individuals always appear to be involved in its production.

The actual fusion between two thalli in the same coenobium has not been watched. The earliest stage which I have observed is illustrated in Text-fig. 3. Here two thalli have fused to form a common thallus. Three of these fairly early stages have been seen. In the fusion cell it is impossible to decide with certainty how much of it originally belonged to one thallus and how much to the other. It is, therefore, impossible to say whether the process is isogamous or anisogamous, but my impression, based in the examination of a large number of fusion thalli attached to zygospores, is that it is probably isogamous. From the fusion cell the zygospore is developed as a bud into which the protoplasmic contents pass. In association with the empty thallus, attached to each zygospore, are two zoospore knobs on the surface of the coenobium (Text-fig. 4, J, K, L, M, N and Pl. 2, no. 2). These



Text-fig. 4. *Endocoenobium Eudorinae*. Mature zygospores. On each empty fusion-thallus two zoospore knobs can be seen. These are on the surface of the coenobium. In J and K only about half of the host cells are parasitized. In L, M and N most of the host cells of the coenobium are diseased. In N, so as not to obscure the fusion-thallus, the outlines of the diseased cells are dotted. Drawn with camera lucida from living material. ×650.

represent the two original zoospores from which the two thalli developed which fused prior to zygospore formation. More than fifty examples of this stage have been examined. Sometimes the zoospore knobs are quite close together, but frequently they are at opposite poles of the coenobium and it is usually impossible to get them in focus simultaneously. In the case shown in Pl. 2, no. 2, one of the zoospore knobs is clearly to be seen while the other is just out of focus.

It is interesting to note that the zygospore, as far as position is concerned, bears much the same relationship to the fusion thallus as the zoosporangium does to its thallus, but whereas the zoosporangium projects from the host coenobium, the zygospore is formed within the coenobium.

The formation of a zygospore as an outgrowth from a fusion cell has not, so far as I know, been reported in the Chytridiales, but it is well known in other Phycomycetes such as *Syncephalis nodosa* and *Entomophthora americana* (Gwynne-Vaughan & Barnes, 1938).

The zygospore has a fairly thick wall covered with small spines and contains numerous oil drops. It varies considerably in size (15–30μ) and the variations appear to depend to some extent on the size of the *Eudorina* which is parasitized. Relatively large zygospores are produced in large coenobia and relatively small ones in small coenobia. So far attempts to germinate the zygospores have failed.

AFFINITIES OF *ENDOCOENOBIUM*

There seems little doubt that of all the members of the Rhizidiaceae *Endocoenobium* most closely resembles *Polyphagus*, a genus founded on the single species *P. Euglenae* (Nowakowski, 1876; Wager, 1913).

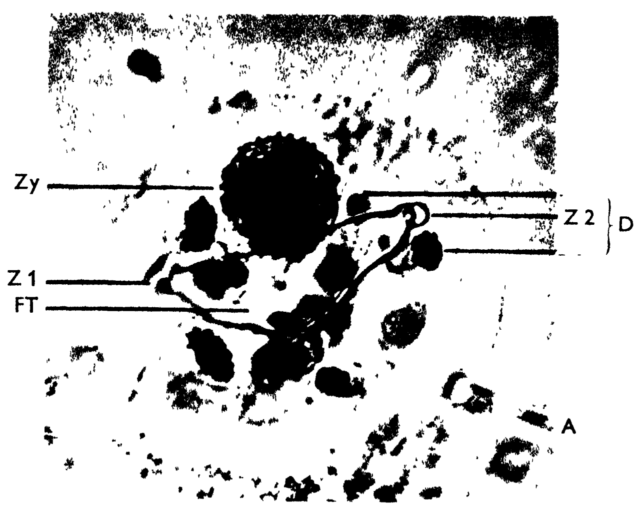
It is in its asexual stage that *Endocoenobium* shows its closest resemblance to *Polyphagus*. As in *Polyphagus* the thallus taps a number of host cells by means of branched and unbranched rhizoidal outgrowths. In both genera the zoosporangium is developed as an outgrowth from the thallus. In size, form and dehiscence the sporangium is very much alike in the two genera. The chief difference is that whilst in *Polyphagus* the encysted zoospore enlarges directly into the thallus, in *Endocoenobium* the encysted zoospore remains as a knob on the surface of the parasitized coenobium, and the thallus forms within the coenobium as an outgrowth from this knob. Dr Sparrow has drawn my attention to the fact that this difference is clearly related to the difference in the habitat of the two fungi. *Polyphagus* attacks encysted *Euglena* cells which are free from one another, while *Endocoenobium* has to penetrate the tough coenobium wall before it can tap the host cells.

It is in the sexual stage that *Endocoenobium* differs most markedly from *Polyphagus*. Indeed the resemblance lies only in the fact that a thick-walled zygospore is produced in both genera as the result of a sexual process.

OTHER CHYTRIDIALES PARASITIZING *EUDORINA*

Endocoenobium Eudorinae is not the only chytridiaceous parasite known to occur on *Eudorina elegans*. In Swithland Reservoir I have seen *E. elegans* parasitized by *Dangeardia mamillata* which Schröder (1898) originally described on *Pandorina*

No. 1



No. 2

INGOLD—*ENDOCOENOBIUM EUDORINAE*

morum. Again Cook (1932) described the minute chytrid, *Rhizophydium transversum* as attacking *Eudorina elegans* in Britain.

SUMMARY

Endocoenobium is a new genus of the Chytridiales in the family Rhizidiaceae and the sub-family Polyphageae. *E. Eudorinae* is the only species and this grows as a parasite on *Eudorina elegans*.

The sac-like thallus develops within the host coenobium and rhizoidal outgrowths make contact with a number of cells.

Asexual reproduction is by uni-ciliate zoospores produced in an elongated zoosporangium which projects from the *Eudorina* coenobium.

A spiny, thick-wall resting spore (zygospore) is produced as the result of the sexual process.

Endocoenobium is considered to agree more closely with *Polyphagus* than with any other genus of the Rhizidiaceae.

My thanks are due to Dr F. K. Sparrow, to whom I sent my photographs and figures, for his kindness in giving me the benefit of his opinion. I have also to thank Mr N. Reynolds, who has been investigating the organisms of Swithland Reservoir, for supplying me with samples of fresh plankton for this study.

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EXPLANATION OF PLATE 2

Endocoenobium Eudorinae

No. 1. *Eudorina* coenobium with the asexual stage of the fungus. *T*, thallus; *Z*, zoospore-knob; *S*, zoosporangium; *H*, healthy *Eudorina* cell; *D*, diseased *Eudorina* cells; *A*, parts of two cells of an *Asterionella* colony which happened to lie beside the diseased *Eudorina*. Photographed in the living condition. × 705.

No. 2. *Eudorina* coenobium containing the sexual stage of the fungus. *Z* 1, one zoospore-knob (out of focus); *Z* 2, other zoospore-knob (in focus); *FT*, fusion-thallus; *Zy*, zygospore; *D*, diseased *Eudorina* cells; *A*, part of an *Asterionella* cell which happened to lie nearby. Photographed in the living state. × 705.

FURTHER STUDIES OF THE INFLUENCE OF LIGHT UPON THE WATER INTAKE AND OUTPUT OF LIVING PLANT CELLS

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(With 10 figures in the text)

I. Introduction

Our previous investigations into the problem of the light permeability reaction have led us to conclude that the water permeability of light-sensitive plant tissues is increased by illumination. The first evidence for this fact was derived from our analysis of the phototropic reaction of the pulvinus of *Phaseolus multiflorus* (M. Brauner, 1932). Later, a renewed investigation of the problem showed us that some simple storage tissues (*Daucus*, *Beta*) react in a way similar to that of the specialized motor organs, i.e. the water intake of the wilted parenchyma—under otherwise constant experimental conditions—appeared to be accelerated by strong white light (L. Brauner, 1935*b*; L. and M. Brauner, 1936).

To make things clear we must first consider several physical facts concerning the structure and behaviour of the membranes to be passed by the inflowing water. There is little doubt that the path available for the entrance of water into the cell is the pores and the intermicellar spaces of the latter's dense boundary layers. So it would be natural to assume that the velocity of the flow is governed by the actual width of these spaces as in an ultrafilter. Recent research, however, has rendered it very probable that filtration processes of the kind with which we have to deal are also subject to another factor of not less importance: the electrical interrelation between the walls of the pores to be passed and the moving particles themselves. From the electrical behaviour of our experimental objects when inserted as membranes in a concentration chain it can be safely concluded that, under biologically normal *p*H conditions, the responsible boundary layers must be negatively charged relative to the liquids in contact with them. Therefore the passage of corpuscles, which are themselves carriers of an electric charge, will be influenced by the electric field between them and the walls of the pores. This effect, the importance of which was first recognized in the diffusion of ions through dense membranes, ought also to affect the movement of water molecules, since their dipole-character would, in spite of their electroneutrality, render them subject to the charge of the pore wall.

In which way can illumination influence these factors? According to one of the main principles of photochemistry the primary effect of light in photosensitive systems consists in a separation of electrons from the absorbing surface, which in its turn implies a loss of negative charge by the irradiated body. If the system, like our membranes, was originally negatively charged, then this loss must mean a more

or less far-reaching discharge of the pore structure. This in its turn would be liable to affect the passage of water and solutes *directly* in two ways, differing from each other in principle:

(*a*) Since a discharge of the micellae building up the boundary layers must lead to a reduction of their mutual electrical repulsion, their cohesion forces will prevail and cause a contraction of the illuminated structure and a narrowing of the pores and intermicellar spaces. In two previous papers (L. and M. Brauner, 1936, 1938) evidence was given for the actual occurrence of such reactions. In the investigation cited first we found the exosmosis of mono- and disaccharides from living carrot parenchyma to be considerably reduced by light; and in the second an analysis of the photoelectric reactions in membrane models and living tissues led us to conclude that the mobility of cations, and of at least one very voluminous anion (PO_4'''), must be considerably decreased within the illuminated boundary layers of these systems.

(*b*) The behaviour of water, however, was not compatible with this conception. As its osmotic intake was proved beyond doubt to be increased by illumination, the constriction of the pore diameter of the diffusion path must have been over-weighted by a second, accelerating effect. Until now the nature of this factor could be interpreted only on theoretical grounds. In principle three entirely different possibilities had to be taken into account: (1) an increase of the osmotic capacity of the tissue which, according to the classic suction force equation, could have its cause either in a rise of the osmotic value of the cell content, or in a decrease of the wall pressure (E. Bünning, 1939, p. 169; E. Heitz, 1925); (2) a decrease of the friction to which the water threads are subject when moving through the pores of the membrane. Since, for the reasons given under (*a*), the change of the pore width to be expected in the illuminated system would act in just the opposite sense one would have to think rather of a decrease of the electrostatic braking forces of the pore walls after their partial discharge. In the event of the available pore diameter being large in comparison with the size of the diffusing dipole molecules this accelerating factor might more than compensate the hindering effect of the pore constriction. In our last paper (L. and M. Brauner, 1938) some evidence was given that the light reaction of *small* anions in photosensitive membranes could be understood best on the grounds of this theory; so its application to the still smaller water dipoles does not seem totally unfounded. (3) The third possibility to be considered is the participation of an electro-osmotic process. Any difference in the concentration of the cell sap of the tissue and its surrounding liquid medium must lead to the establishment of a diffusion potential the magnitude of which will be determined by the existing concentration gradient, by the mobility of the ions concerned, and by the properties of the separating membranes. J. Loeb (1920–1) in particular has been able to demonstrate that the resulting electric field across the diaphragm is bound to cause a water movement in the pores of the latter, the direction of which depends on the sign of the electric charge of the pore walls and of the liquids on the two sides of the membrane—anomalous osmosis. This additional force must obviously interfere with the *normal* osmotic suction force of the tissue and, according

to its direction, accelerate or retard the velocity of the water flow. Since according to our previous experiences pre-existing membrane potentials are affected by illumination—if the diaphragm is light sensitive—light may influence the water intake *indirectly* by modifying the electro-osmotic component of the tissue. From our former studies we are now able to predict the direction of the forces and reactions involved: submerged in distilled water the tissue will, owing to its comparatively higher cation permeability, render the surrounding medium positive. The pore walls of the boundary layers being electronegative, the water threads crossing them must be attracted by the negative "pole" of the concentration chain, viz. the interior of the tissue. So in this system the normal endosmosis of water will be furthered by the similarly directed electro-osmotic flow. If the original potential difference of the chain is now enhanced by light—as in the case of the previously studied *Helodea* leaf and some membrane models—then the water inflow would appear to be accelerated, in the same way as after a mechanical increase of the water permeability.

The three mechanisms discussed would finally differ essentially in their equilibrium conditions, the understanding of which may help us later in the critical analysis of our experimental results. In our first case (increase of the normal suction force by illumination) the light would alter a static quality of the cells, affecting not only the velocity of the water intake, but also the total gain when the process has been completed. In graphic representation the saturation curve of the illuminated tissue would consequently not only rise more steeply than the corresponding "dark" curve, but also remain above the latter up to its asymptote (Fig. 1 *a*). In the second case (decrease of the electrostatic friction within the pores) the light reaction would only concern the dynamic velocity-factor of the water intake, and would leave the static end-conditions unaltered. So the "light" curve would again rise more steeply, but then sooner or later converge with the "dark" curve, so that their asymptotes would eventually coincide (Fig. 1 *b*). In the third case (light-influenced electro-osmotic component) the dynamic effect of the reaction would again be the same as in the previous examples, but the further course of both graphs would necessarily depend on the permeability of the tissue to the electrolytes responsible for the diffusion potential. In the theoretical case of absolute impermeability, the additional electro-osmotic component would persist until a static end state, comparable to a Donnan-equilibrium, had been reached (Fig. 1 *c*). If, however, the system loses ions by exosmosis during the process of water intake, then the additional component would gradually decrease until only the normal osmotic force would be left in control of the final equilibrium (Fig. 1 *d*).

It must be emphasized, though, that all these considerations are true only on condition that the system in question actually approaches a *static* equilibrium, an assumption which may by no means be taken for granted.

In order to permit a decision between the different possibilities discussed above, the experiments of the present investigation had to be planned according to the following scheme:

(*a*) The water intake by illuminated and darkened tissue had to be observed

over a period sufficiently long to indicate the general trend of the saturation process.

(*b*) It has to be investigated whether, and in which direction, the "normal" light reaction could be influenced by ions in the external medium, the presence of which would alter the original diffusion potential between tissue and medium in a predictable way.

(*c*) Since the participation of an electro-osmotic component in the photo-reaction must give polarity to the latter, it had to be investigated what character

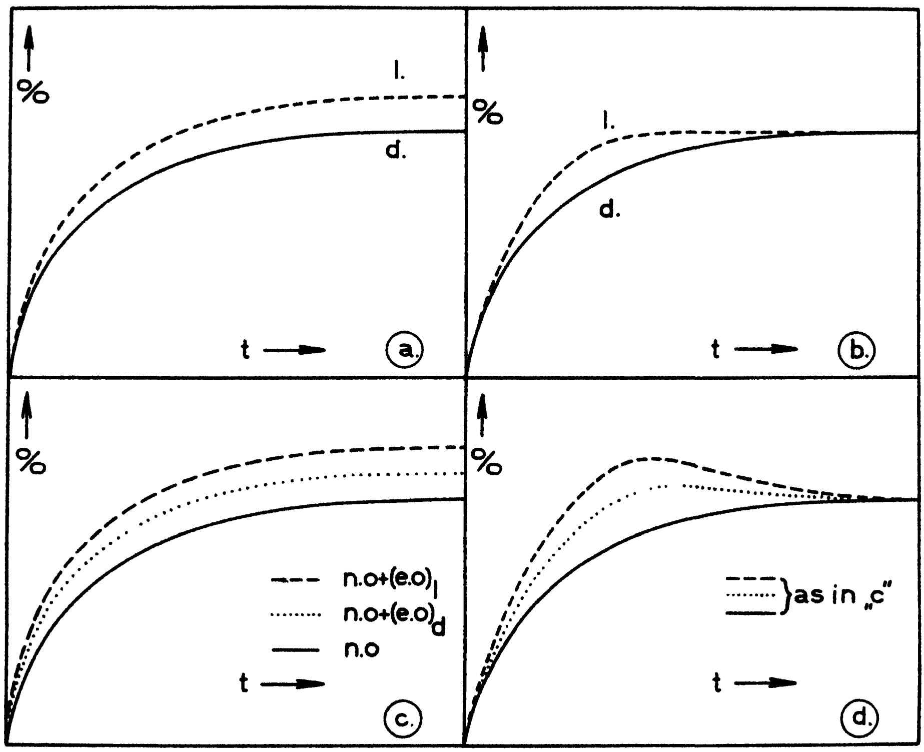

Fig. 1. *l* = water intake in light, *d* = water intake in darkness, *n.o.* = normal osmosis, *e.o.* = electro-osmosis. Ordinates: % = percentage gain in weight. Abscissae: *t* = time. For (*a*)–(*d*) see text.

the light effect would assume if the direction of the water exchange were reversed by employing hypertonic sugar solutions as the external medium.

All these experiments were based on the following technical principle: a homogeneous quantity of a suitable light-sensitive tissue was cut up into small disks and divided into two identical groups. These were weighed and then, in order to secure dark adaptation, kept for a certain period in a light-tight incubator. After this preparation time the samples were transferred to two containers, filled according to the kind of experiment, with water or one of the above-mentioned solutions. One sample was kept darkened, and the second one subjected to intense illumination, during which great care had to be taken to keep the temperature in both

receptacles as similar as possible. After the fixed time of exposure both lots were weighed again; from the observed changes the effect of light upon the water exchange between tissue and external medium could be deduced.

II. MATERIAL AND METHOD

As experimental material we used throughout this investigation the root tissue of winter carrots, which has already, during our previous work on this subject, proved its suitability for the present purpose.[1] The local variety of *Daucus carota* chosen is characterized by roots of from 20 to 25 cm. length and about 5 cm. maximum thickness near the upper end. The anatomical structure of the tissue

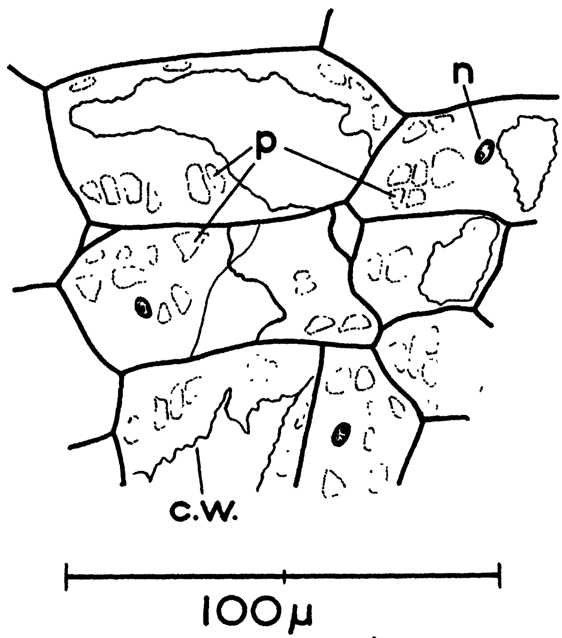

Fig. 2. *Daucus carota*. Radial longitudinal section through the phloem parenchyma of the root. *p* = simple pits, *n* = nucleus, *c.w.* = cell wall cut open. (Camera lucida drawing from 10μ microtome section stained with iron haematoxylin.)

corresponds more or less to the description given by J. S. Turner (1938). Only one interesting detail requires additional mention: the parenchyma-cells of the phloem, especially at their radial-longitudinal walls, show huge irregular pits (Fig. 2), a fact which may be of importance in our experiments for the speedy translocation of water through the tissue.

From the upper part of the root transverse disks of about 5 cm. thickness were taken; from these, 8–10 cylinders were cut in a longitudinal direction around the cambium-zone with a 8 mm. cork-borer. The cylinders accordingly contained more or less equal amounts of the pale yellow xylem and the orange-red phloem tissue.

[1] The possible role of the carotenoids of the tissue as light-sensitizers has already been pointed out in our last paper (L. & M. Brauner, 1938, pp. 203, 238, 245).

By means of a Gillette blade and a suitable brass pattern of 8 mm. bore and 5 mm. height these cylinders were then sectioned to disks of 5 mm. thickness, the total surface of each of which consequently amounted to *ca.* 250 mm.[2] This material was finally well mixed and equally distributed into two closable weighing glasses. Both samples, which contained 30–33 pieces each, were weighed upon an analytical balance and then, enclosed in a lightproof box, kept for 40 min. in an incubator at 25° C. Meanwhile in an adjoining darkroom an electrically heated glass water-bath of 10 l. content was adjusted to the same temperature; into this tank two Erlenmeyer flasks of 200 ml. content were immersed, each filled with 150 ml. of the experimental liquid. One of these flasks was made impervious to light by a double coat of paint, an internal black one, and on top of it, one of aluminium bronze to

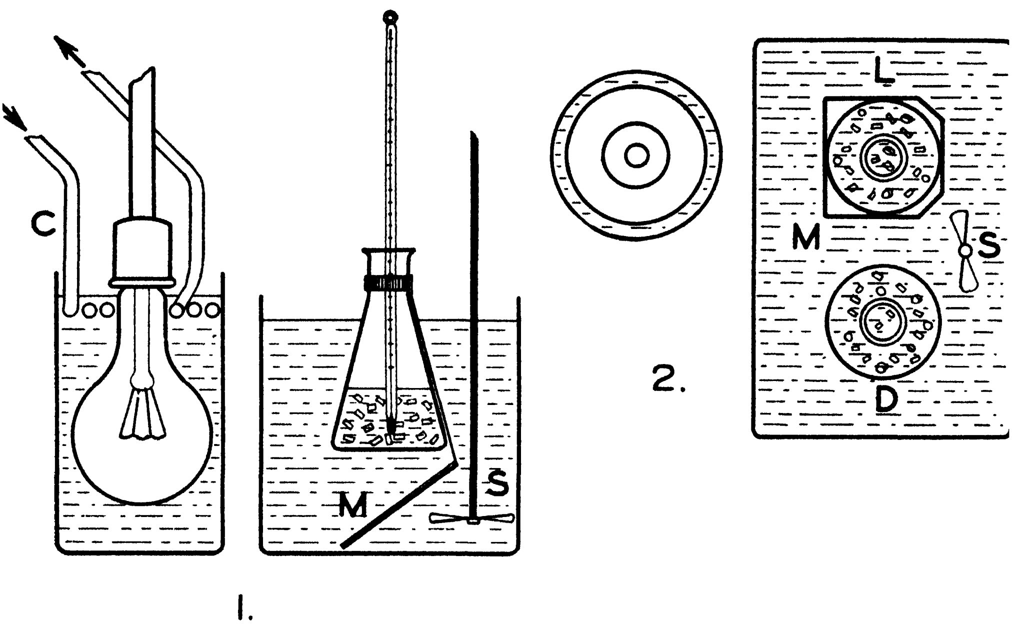

Fig. 3. Experimental arrangement. (1) side view, (2) top view. L = transparent container, D = darkened container, M = mirror, S = stirrer, C = cooling spiral.

avoid heating up under the radiation of the high-power incandescent lamp used in the experiments. The second flask was left transparent, and a mirror was fixed obliquely below it so as to reflect the light coming from the side in the direction of the bottle's axis.

Outside the tank a 500 W. Tungsram lamp was fixed inside a glass cylinder filled with distilled water and covered with a cooling spiral through which cold tap water circulated. By this arrangement the development of heat could be reduced to a minimum. Both Erlenmeyer flasks were fixed side by side in the centre of the bath, the central distance between the clear bottle and the filament of the lamp being 20 cm. The temperature in each container could be continuously observed with a decigrade thermometer. An electric stirrer secured a very uniform heat distribution throughout the tank (Fig. 3).

To start the experiment the two weighed samples of tissue were simultaneously put into the Erlenmeyer flasks; and immediately afterwards the strong electric lamp was switched on. After the end of the illumination period both flasks were taken out of the bath and their whole content quickly poured into two porcelain sieve funnels. From them the tissue disks of both samples were collected, carefully freed from all adherent water with soft filter paper, and finally transferred to their respective weighing glasses. A second weighing then showed the water exchanges of the darkened and illuminated samples. All manipulations before and after the illumination period were performed in weak yellow light.

III. Qualities of the light source used

For the present investigation two types of high power incandescent lamp were used: a so-called "episcope" lamp, with the filament arranged in the vertical plane; and after its accidental breakdown a normal 500 W. lamp with ring-shaped filament.

The lamp was in series with a suitable regulating resistance and an ammeter connected to the A.C. mains. Since our laboratory is supplied with electricity from a special transformer station the fluctuations of the tension were, especially during the hours of our experiments, remarkably low ($\pm 1\%$). With our normal voltage of 112 V. the energy uptake of both lamps was the same, 506 W.

In order to measure the intensity of the light field at the locus of the experimental objects a carefully blackened thermocouple connected to a sensitive mirror galvanometer was inserted into the transparent Erlenmeyer flask. Under our normal experimental conditions with the lamp cooled, the instrument indicated an energy uptake of 36×10^{-3} cal./cm.^{2}/sec. in the centre of the immersed container. The total thickness of all water layers to be passed by the light from lamp to object amounted to 8 cm.; that of the glass walls (water tank, and lamp cylinder) to 11 mm. So it can be computed that the transmitted radiation must have comprised the wave-length band from *ca.* 350 mμ up to *ca.* 800 mμ with the centre of gravity probably around 700 mμ.

IV. Control experiments

(1) *Degree of reliability of the method employed*

Our preliminary experiences showed that the absolute differences to be expected between the water exchange of the light and the dark samples would, even under optimum conditions, be but small, viz. of the magnitude of from 50 to 70 mg. for samples of 8–9 g. fresh weight. So, before far-reaching conclusions could be drawn from such figures the dependability of the method employed had to be carefully tested. For this purpose a set of blank experiments were performed, in which the water absorption of both tissue samples took place under identical conditions, viz. in complete darkness. The technical procedure corresponded exactly to that followed in our main experiments. The tissue disks used for each pair of determinations were taken from the same carrot. The experimental temperature was 25·0° C., the exposure time 20 min. The whole series comprised, like those of the

light experiments, twenty pairs of determinations. The result was calculated according to the same principle that was applied later in the main experiments: since the water intake of the two parallel lots could be directly compared only within each double experiment, the ratio of the percentage water gains $a\%/b\%$ had to be worked out separately for all co-ordinated samples; the arithmetic mean of the whole series was then calculated from the twenty ratio figures obtained, which for convenience were multiplied by 100. In this method equality of water intake in both sets would be expressed by the figure 100·00. To complete the result the standard deviation of the arithmetic mean $\sigma_m = \sqrt{\frac{\Sigma pd^2}{n(n-1)}}$ has been added in each case.

The issue of the whole series approached the ideal value of 100·00 quite closely:

$$1/20\ \Sigma\ (100 \times a\%/b\%) = 99{\cdot}59 \pm 0{\cdot}97.$$

Writtėn in absolute figures this ratio would indicate a weight difference of 2·6 mg. between the water gain of the two samples. This, then, was the magnitude of the uncertainty factor, due to unavoidable differences in the after treatment of the material such as drying, which we had to take into account in the later main experiments. It amounts, as we shall see, to *ca.* 0·04 of the maximum light effect met in the present investigation.

(2) *The effect of temperature upon the water intake*

The illumination of a system which absorbs the incident radiation will necessarily raise its temperature, an effect which no filtering off of heat rays can prevent. Consequently in our experimental arrangement a temperature difference must have developed between the contents of the transparent and the darkened flask. In order to reduce this difference to a minimum, as much as possible of the local rise of temperature had to be led off and redistributed by immersing the containers in a sufficient quantity of well-stirred water. In experiments with prolonged illumination time (60–120 min.) keeping the room temperature well below that of our thermostat proved very helpful. As a result of these measures the temperature difference between the two containers actually never exceeded 0·2° C. in the average of a set of twenty single experiments, usually it was considerably lower. Though these figures may seem small, we thought it necessary to measure the temperature coefficient of the water intake of our experimental object, since at least three factors of the process could be expected to possess a $Q_{10} > 1{\cdot}00$, viz. (*a*) the osmotic value of the cell-sap, (*b*) the water permeability of the boundary layers, and (*c*) the fluidity of the moving water. Such a special determination seemed the more necessary since the existing figures on this subject reveal a surprising discrepancy. To that end our experiments were performed in the following manner: two water-tanks of 12 l. capacity were adjusted to the temperatures to be compared: (*a*) 10 and 20° C.; (*b*) 20 and 25° C.; (*c*) 25 and 30° C. In each bath one Erlenmeyer flask of 250 ml. capacity was immersed and filled with 150 ml. of distilled water carefully brought to the same temperature. These two containers were then charged

with corresponding weighed lots of carrot disks cut from the same root (*ca.* 9–10 g. per bottle). This procedure caused a very slight fluctuation of the temperature (magnitude 0·1–0·2° C.) which, however, never lasted longer than 1 min. The time of exposure chosen was purposely rather short, viz. 20 min., in order to remain sufficiently far from the saturation state of the tissue to include only the first and most energetic part of the reaction, which probably would be more influenced by temperature than the static equilibrium. The experiments were performed in complete darkness.

After the fixed time the material was removed from the receivers, dried and weighed again as described above. Each temperature range was tested five times with tissue taken from different plants.

From the figures obtained the temperature coefficient of the water intake was calculated by comparing separately the percentage gain in weight of each corresponding pair of determinations.

Table 1. *Temperature effect upon the water intake of carrot tissue in darkness. Each value is the average of five determinations. Exposure time:* 20 *min.;* 4–18 *November* 1938

Range of temperature ...	10–20° C.	20–25° C.	25–30° C.
% water intake $t_2/t_1 \times 100$	111·24	104·00	105·34
Q_{10}	1·1124	1·0800	1·1068

Compared with the findings of earlier authors these values appear very small. E. M. Delf's (1916) much-cited Q_{10} figures vary between 1·4 and 3·8 (water exosmosis from *Allium* leaves and *Taraxacum* scapes); Iz. de Haan (1933) calculated from his measurements of the rate of deplasmolysis Q_{10} values of *ca.* 2·7 (20–30° C.) for the water permeability of the onion scale epidermis. Somewhat smaller figures are given by W. Stiles & I. Jørgensen (1917) in their paper on the water intake of carrot tissue, i.e. our own experimental object:

$$Q_{10\text{–}20^\circ\,\text{C.}} = 1{\cdot}3; \quad Q_{15\text{–}25^\circ\,\text{C.}} = 1{\cdot}4; \quad \text{and} \quad Q_{20\text{–}30^\circ\,\text{C.}} = 1{\cdot}6.$$

It is worth noting that the same authors, using the same method, found much higher values with another tissue, potato parenchyma ($Q_{10\text{–}20^\circ\,\text{C.}} = 3{\cdot}0$, $Q_{20\text{–}30^\circ\,\text{C.}} = 2{\cdot}7$). So our object in those experiments also seems to have been distinguished by a remarkably low temperature coefficient.

In comparing our own results with the figures given by Stiles & Jørgensen, it must be borne in mind that our findings demonstrate the temperature effect upon the water intake as a whole and *not* upon the water permeability alone. In order to find the latter one had to take into account first the respective losses of suction force during the 20 min. of water intake, which naturally must have been greater at the higher temperature, second the changed viscosity of the water itself, and third the temperature effect on the water capacity of our tissue. Consideration of the first of these three factors alone would obviously cause the permeability reaction to appear greater than the observed absorption ratios; on the other hand taking

into account the second and third factors would make the permeability reaction appear less. As Stiles & Jørgensen did consider the positive component, but disregarded the two negative ones, their figures must necessarily appear higher than the true Q_{10} values of the water permeability.

From the magnitude of our temperature coefficients some facts may be inferred as to the nature of the absorption process in question. Values considerably below 2·00 are known to be characteristic of purely physical processes. So the osmotic potential which rises proportionally to the absolute temperature is (between 10 and 20° C.) subject to a Q_{10} of 1·035. The temperature coefficient of the fluidity $(1/\eta)$ of pure water has the value of 1·29 between 10 and 20° C., and 1·26 between 20 and 30° C. These data are calculated from the viscosity figures cited in Hatschek's monograph (1929, p. 66). The effect of temperature on most diffusion processes seems to be of the same order. The Q_{10} values above 2·00 which are not infrequently mentioned in the literature, would indicate that chemical processes rather than physical ones were involved in the relation of temperature to water intake (cf. Stiles, 1924, p. 147). Such changes, however, are more likely to concern the suction force of the tissue than its permeability to water. One could also consider, as did Iz. de Haan (1933, p. 303), a modification of swelling processes, which might influence both the suction force and the state of the boundary layers, viz. their permeability itself. A misconception has to be avoided, however, to which the last mentioned author seems to have succumbed. Most swelling phenomena are exothermic processes, the final equilibrium of which must consequently be expected not to rise but to fall with increasing temperature (cf. H. Freundlich, 4th ed., **2**, 584). The velocity, however, with which this final state is approached depends directly on the temperature and seems, within certain limits, to follow Van't Hoff's rule ($Q_{10}=2$–3). From these conditions it must be concluded that only the *transition time* from a lower to a higher degree of swelling can be subject to the above mentioned high Q_{10} values, but never the final equilibrium of the process itself. As a longer discussion of these important problems would exceed the scope of the present paper a thorough analysis of the matter has to be left to a future investigation. For our present purpose it may suffice to have shown that the compound Q_{10} value of all factors determining the water intake of our material was, at the temperature range of our experiments, of the order of $\sim 1{\cdot}1$. This finding implies, for our light experiments, that an absorption surplus of at most 0·2% in the illuminated sample has to be attributed to a simple temperature effect.

V. The photoreaction in distilled water with different times of exposure

It was the aim of our first main experiments to follow up the development of the light reaction during the time of exposure. The whole series comprised six sets of measurements ranging from 5 to 120 min. Before, however, the results of the light effect are set forth it seems necessary to give a short summary of the general trend of the process of water intake in our tissue. From preliminary experiments

it soon became clear that, of all the qualities of the material concerning our problem, the *absolute* speed of the water absorption showed the highest degree of individual variability. As the suction force of the tissue proved to be remarkably constant (cf. Table 9), the varying factor must have been the water permeability of the cells. This fact finds its expression chiefly in the shape of the initial rise of the saturation curves, the steepness of which varies much more with the different samples than does their subsequent phase, which seems to approach a static equilibrium. So in order to give a more consistent impression of the trend of the process the water intake was measured in a special series with six lots of *identical* tissue per experiment, according to the following scheme:

Lot no. ...	1	2	3	4	5	6
Time of exposure, min.	5	10	20	40	80	160

The experiments were performed in complete darkness in a thermostat at 25·0° C. and repeated five times. The following Table 2 shows the mean result of these five independent measurements.

Table 2. *Water intake of cylinders of carrot tissue*, 8 × 5 *mm.*, *in darkness at* 25·0° C. *Mean values of five experiments.* 6–9 *December* 1938

Exposure time, min.	5	10	20	40	80	160
Gain in % of original weight	7·94	9·66	11·59	13·09	14·48	15·91

A graphic extrapolation from these figures would indicate an equilibrium at *ca.* 16·5% after *ca.* 5 hr. (cf. Fig. 4), a state which, however, is in reality never reached, because physiological changes within the tissue as yet unknown lead to a resumption of the water intake which may last for days. This experience, which confirms similar findings by D. E. Reinders (1938), implies an important consequence for our light experiments: if the tissue for any metabolic reasons never saturates its suction-force completely, then no final coincidence can be expected between the "light" and the "dark" curve, because under these conditions the process of water intake would not approach a *static* equilibrium.

The general result of the six main series of our "light"/"dark" experiments are given in the following Table 3. It should be remembered that the figures again show the mean ratios of the percentage water gain of the two co-ordinated samples,

Table 3. *Light effect upon intake of distilled water. Light intensity* 36×10^{-3} *cal./cm.²/sec. Initial temp.* 25·0° C.

Time of exposure min.	Final temp. diff. light-dark °C.	$100 \times \frac{\% \text{ light}}{\% \text{ dark}} \pm \sigma_m$	Mean		Mean	
			$-\sigma_m$	$+\sigma_m$	$-3\sigma_m$	$+3\sigma_m$
5	+0·050	102·0 ± 0·997	101·00	103·00	99·01	104·99
10	+0·110	104·0 ± 0·772	103·23	104·77	101·68	106·32
20	+0·165	106·5 ± 0·691	105·81	107·19	104·43	108·57
30	+0·200	108·3 ± 1·061	107·24	109·36	105·12	111·48
60	+0·150	107·0 ± 0·824	106·58	108·22	104·93	109·87
120	+0·145	105·2 ± 0·842	104·36	106·02	102·67	107·73

viz. $100 \times \%$ gain of the light sample/% gain of the dark sample. Each point was repeated twenty times with different materials. To allow for a judgment of the range of dispersion of the results, the single and triple standard deviation of the arithmetic mean has been indicated for each value (cf. Fig. 5). Fig. 5 shows the total result of the whole series in graphic representation, in which the $100 \times \%$ "light"/% "dark" values are plotted against time within the field of their single and triple standard deviations. We notice that the photoreaction consists in a furthering of the water intake over the whole tested range. This effect appears as a distinct maximum function of the exposure time; it reaches its peak in a steep rise at the 30 min. point, after which a comparatively gradual decline follows.

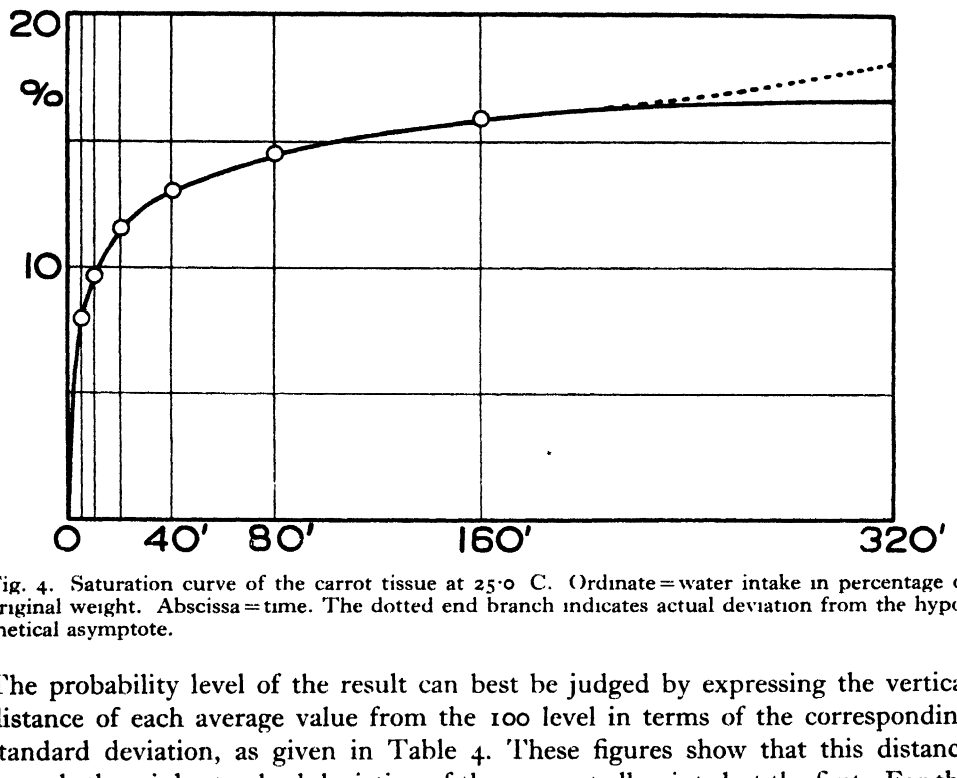

Fig. 4. Saturation curve of the carrot tissue at 25·0 C. Ordinate = water intake in percentage of original weight. Abscissa = time. The dotted end branch indicates actual deviation from the hypothetical asymptote.

The probability level of the result can best be judged by expressing the vertical distance of each average value from the 100 level in terms of the corresponding standard deviation, as given in Table 4. These figures show that this distance exceeds the triple standard deviation of the mean at all points but the first. For the 5 min. value the probability of the "true" ratio equaling 100·00 amounts to 45 in 1000, but for all the others it is less than 6 in 100,000.

Table 4. $[100 \times \frac{\%\ \text{light}}{\%\ \text{dark}} - 100] : \sigma_m$

Exposure time, min.	5	10	20	30	60	120
Multiples of σ_m	2·01	5·18	9·40	7·82	8·98	6·17

We have now to consider what facts may be inferred from the general trend of the whole curve. The most likely explanation of the ascent up to the peak seems to

be the assumption that, under the described experimental conditions, the photoreaction needs about 30 min. to develop to its full height. More difficult, however, is the interpretation of the subsequent fall of the ratio. At first sight one might accept it as an indication that the reaction is essentially a dynamic one, i.e. that it influences only the friction of the entering water, because the decrease in the quotients coincides with the progressive saturation of the tissue. But an elementary consideration shows that such a conclusion would not be fully justified. Let the water gain of the "dark" sample be d and that of the "light" sample l, equal to

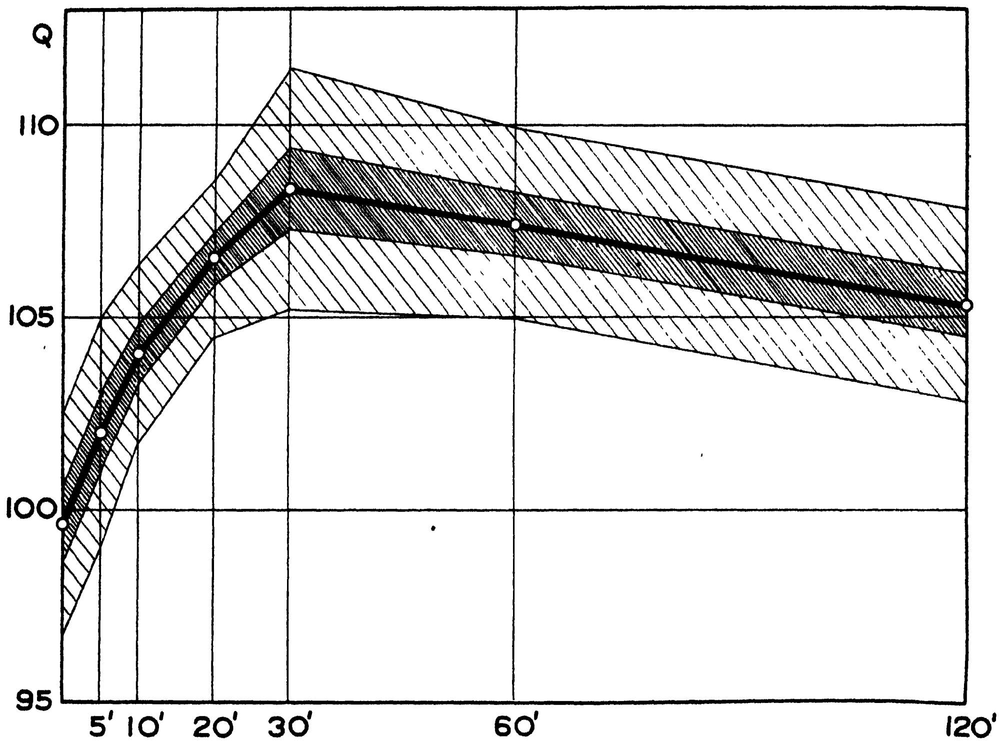

Fig. 5. $Q = 100 \times \frac{\text{percentage water intake "light"}}{\text{percentage water intake "dark"}}$, as a function of exposure time. Hatched fields: heavy = single, light = triple standard deviation of the arithmetic mean.

$d+n$. Then our quotient Q can be written: $\frac{d+n}{d} = 1 + \frac{n}{d}$. Let us now suppose that the vertical distance n between the "dark" and the "light" saturation curve in reality does not diminish as a convergence of the two graphs would imply, but that it remains constant. Considering the relation: $Q = 1 + \frac{n}{d}$ we had in this case again to expect a drop of the Q values with progressive saturation.

Another experience, however, seems to be more reassuring: if we plot all single values of Q of each series separately against the corresponding "dark" water intake, no regularity can be discovered in the dispersion of the values, i.e. high Q

aberrants are by no means related to low intake values, as might be expected from the considerations given above; nor is the opposite the case (Fig. 6). This was the main reason which induced us to give preference to the quotient Q as the most

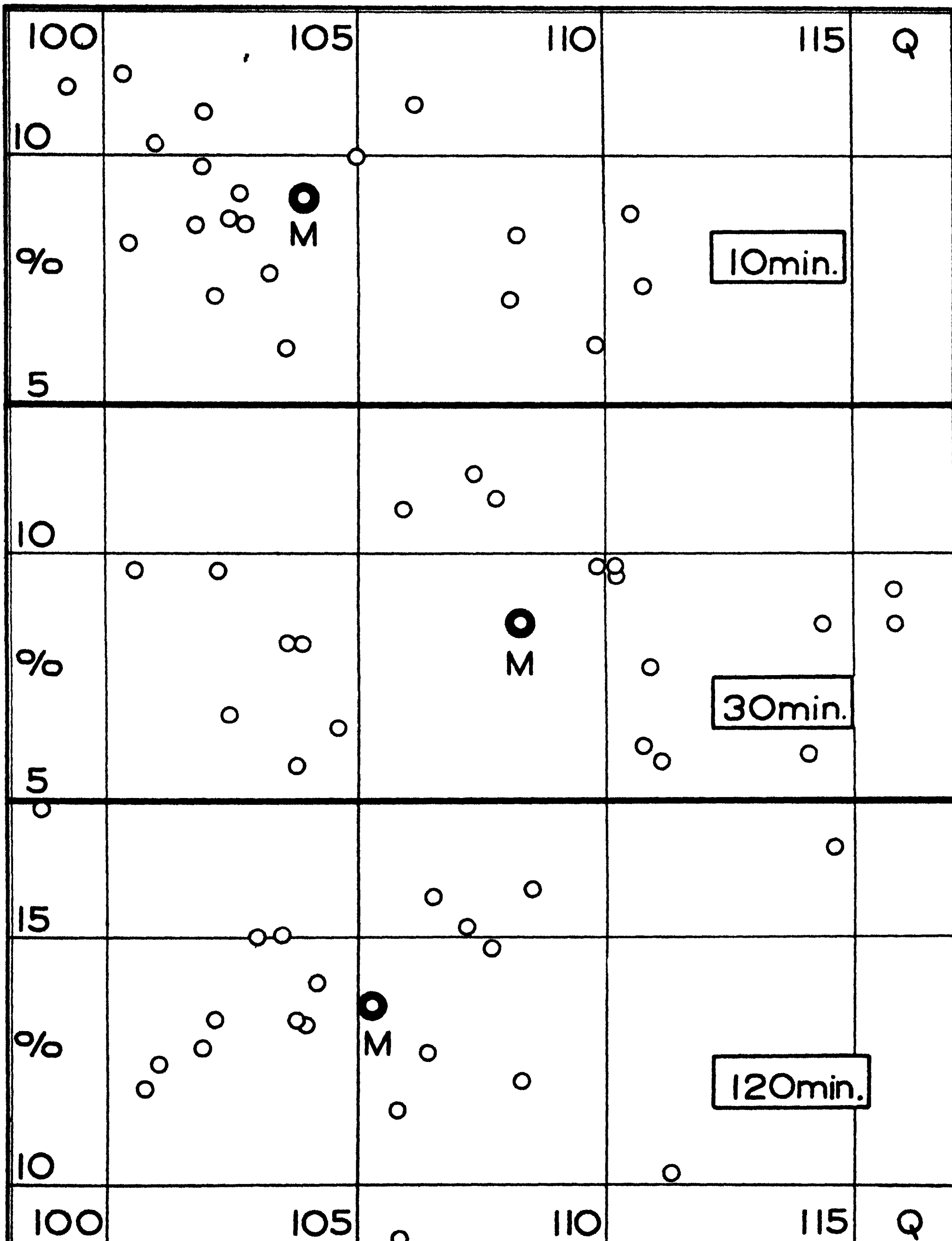

Fig. 6. 10, 30 and 120 min. series. Relation between the dispersion of the water intake percentages and the co-ordinated Q values. M = mean result of each series.

reliable measure of the light reaction. For in our experience the results obtained by ratio-calculation showed much less dependence on the variability of the experimental material than a comparison of the average water intake figures separately computed from all "dark", and all "light" measurements of each series.

In spite of these objections, this latter method of representation will now tentatively be employed to gain some more information about the question of convergence. For this special purpose the 30, 60 and 120 min. figures can be used, since they were obtained with fairly homogeneous material of the same season (January–February). The mean water intake figures of these three double series are given in Table 5.

Table 5

Time of exposure, min.	30	60	120
"Light" water intake %	9·19	12·09	14·36
"Dark" water intake %	8·49	11·23	13·65

If we connect these two groups of points by the two curves of closest fit, an extrapolation towards increasing time seems to indicate a flat convergence, which becomes still more conspicuous, if the intake figures are plotted against logarithmic time abscissae (Fig. 7).

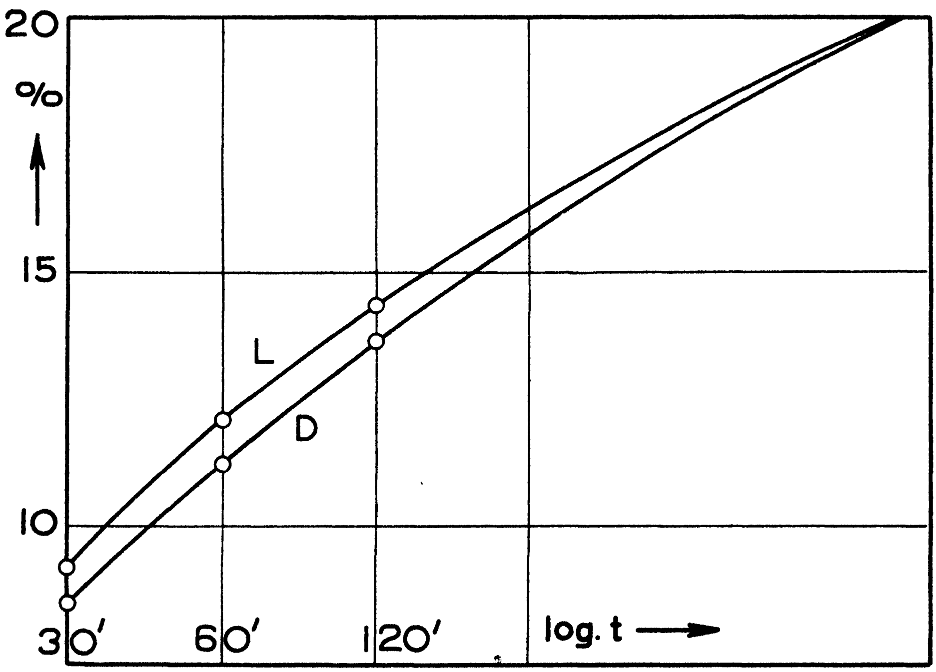

Fig. 7. "Light", *L*, and "dark", *D*, water intake as functions of log exposure time.

It would not be advisable, though, to draw too far-reaching conclusions from such an extrapolation based only upon so few established points. On the other

hand, an experimental verification along broader lines would meet with considerable difficulties, because prolonged exposure times are liable to include the secondary part of the water intake curve. However, we shall be able to show that a definite solution of this question is not essential for the elucidation of the mechanism of the light reaction.

VI. The photoreaction in hypertonic glucose solutions

In the foregoing experiments we have studied the effect of illumination upon the water *endosmosis* into the tissue. In order to decide whether the observed reaction is polar in character it was now necessary to reverse the direction of the water flow by exposing the fully turgescent system to hypertonic solutions of a neutral, non-permeating solute. As such, glucose was chosen in preference to sucrose because of the greater ease with which it penetrates the cellulose walls of the tissue thus establishing a steeper and better defined concentration gradient.

The cut cylinders were infiltrated with distilled water under the air-pump, weighed and, to secure dark adaptation, kept in the light-proof incubator for 40 min. at 25·0° C. After this period the two samples were treated exactly as in the previous experiments, with the only difference that instead of distilled water glucose solution was used. The illumination time was fixed at 30 min., because this period had given the highest effect in the water series. Two different concentrations were tested, 0·5 and 0·7 vol. mol. The total result of these experiments is summed up in Table 6.

Table 6. *Water loss of carrot tissue in glucose solutions. Light intensity: 36×10^{-3} cal./cm.²/sec. Temp.* 25·0° C.

Time of exposure min.	External concentration v.m.	"Dark" loss %	"Light" loss %	$100 \times \frac{\%\ \text{light}}{\%\ \text{dark}}$	No. of experiments pairs	Date
30	0·5	−3·46	−3·45	99·9 ± 2·4	10	22–24. iii. 39
30	0·7	−9·33	−9·32	99·7 ± 1·1	15	25. ii.–3. iii. 39

As the figures show, the water exosmosis is affected by light in neither concentration: the minute differences of the Q values from 100·00 are far less than the corresponding standard deviation of the mean. This unexpected result is contrary to all the possible conceptions of the photoreaction as set forth in the introductory chapter of this paper. For a reduction of the friction within the membrane pores would further the water movement irrespective of its direction, whereas a participation of an electro-osmotic component should involve a decrease of the flow, if, as in our last experiments, the directions of the normal and the electro-osmosis are opposed to each other. Hence, in the first case illumination should increase, in the second decrease, the exosmosis from our tissue; but in none of them was indifference to be expected. So we are led to conclude that contact with the comparatively high concentrations of the glucose solutions employed must have somehow destroyed the light sensitivity of the tissue. This damage may have been

due to the unavoidable densification to which the protoplasmic boundary layers of the cells were subject when in contact with concentrated solutes (cf. L. Brauner, 1935*a*), and secondly to the fact that, especially in the 0·7 mol. solution, the peripheral cell layers of the tissue cylinders were already distinctly plasmolysed after 30 min. immersion.

Since the simple method described proved to be unhelpful in the further analysis of the phenomenon, we had to try to gain new evidence by a direct attack on the pre-existing diffusion potentials between the tissue and its medium.

VII. The photoreaction in hypotonic electrolyte solutions

For the reasons given in the introductory chapter, exosmosis of cell-electrolytes from the tissue must increase the positive potential of the medium. Since the extent of the diffusion potential which arises depends on the concentration gradient of the ions concerned the effect can be expected to reach its maximum in distilled water. The presence of electrolytes in the surrounding liquid must necessarily, according to their nature and concentration, reduce or even reverse this potential difference.

With these conditions in view we thought it promising to investigate the photoreaction of our object in suitably chosen electrolyte solutions. Since the natural cell electrolytes of the carrot tissue account only for a comparatively small part of its total osmotic value it was possible to have the solutions necessary for our purpose so diluted, that the damage to its reactivity which we observed in our glucose experiments could be easily avoided. The technique employed corresponded closely to that of the previous experiments. The chemicals used were Merck's "purissimum" brand, the distilled water necessary for the solutions was prepared with a special Schott glass condenser.

The first electrolyte to be tested was a $N/10$ KCl solution. We chose it because, considering the preponderance of $K^{\cdot}$ among the mobile cations of the cell sap, $K^{\cdot}$ ions in the external medium could be expected to counteract the diffusion potential from the tissue most effectively. The concentration $N/10$ proved to be sufficiently hypotonic to allow a remarkable increase in weight of the immersed tissue. More detailed information on the osmotic properties of the solutions used will be given in the following paragraph. Table 7, which summarizes all our electrolyte experiments, shows in its first column the mean result of a series of twenty such double determinations. As may be seen from these figures the water intake now again appears to be furthered by light. The average value of Q was found to be 107·1, which is 1·3 units less than in the corresponding distilled water experiment. This difference, if rather small, is in the direction originally expected, i.e. it indicates a reduction of the photo-effect. Without further experience, however, we should not yet be justified in concluding from the above result alone that the observed effect was caused by influencing the hypothetical electro-osmotic component. It could go back as well to an osmotic reduction of the photosensitivity of the cells as we have met it in our sugar experiments. For it must not be overlooked, that the applied salt concentration, if hypotonic, still reduces the water intake of the tissue from *ca.* 8·5% (dist. H_2O) to *ca.* 4% (KCl "dark" value).

The question could finally be decided by the application of a sodium phosphate buffer solution as external medium, the remarkable effect of which upon the photoelectric reaction of a living tissue was discovered in our previous investigation (L. and M. Brauner, 1938, p. 225) (Table 7).

Table 7. *Photoreaction in hypotonic electrolyte solutions. Light intensity:* 36×10^{-3} *cal./cm.*^{2}*/sec. Initial temp.* 25·0° C., *final temperature difference* "*l*"–"*d*" ~0·1° C. *Exposure time* 30 *min.*

Solution	*N*/10 KCl	*N*/50 phosphate	*N*/10 phosphate	Distilled water
Water intake "light", %	4·26	8·30	4·49	9·19
Water intake "dark", %	3·98	7·95	4·78	8·49
$100 \times \frac{\%\ \text{light}}{\%\ \text{dark}}$	107·1 ± 1·13	104·2 ± 0·66	93·2 ± 0·96	108·3 ± 1·06
No. of experiments	20	15	20	20
Date	13–17. iii. 39	3–7. iii. 39	8–12. iii. 39	4–12. i. 39

We tested two concentrations both containing NaH_2PO_4 and Na_2HPO_4 in proportion 1:2, giving a *p*H of 6·8. The first one was *N*/50, the second *N*/10 in respect to sodium. The more diluted solution proved so hypotonic that it reduced the water intake only by *ca.* 6·4% compared with pure water. In spite of this low osmotic effect which reaches but 12% of the suction force of *N*/10 KCl, the photoreaction in the buffer mixture appears to be considerably more reduced: $Q = 104{\cdot}2$.

The more concentrated solution, on the other hand, yielded a result quite new in our experiments: the illuminated sample now absorbed *less* water than the darkened one. The corresponding quotient expresses this fact by being less than $100{\cdot}00$: $Q = 93{\cdot}2$ (Fig. 8). Since the standard deviation in both phosphate series is remarkably low (cf. Table 7), the authenticity of this result can be accepted as established: the distance between the Q value and the 100 level corresponds to $7{\cdot}12\ \sigma_m$.

In order to understand the meaning of these results it will be helpful to compare them with our previous experiments concerning the influence of the same electrolytes upon the photoelectric response in organic membranes (L. & M. Brauner, 1938). If a living *Helodea* leaf is inserted as a diaphragm into a concentration chain consisting of *N*/100 and *N*/1000 KCl, light causes the original potential difference across the membrane to *drop*. If, however, the system consists of *N*/100 and *N*/1000 Na-phosphate buffer solution, then the potential difference *rises* under the same illumination nearly to the thermodynamic maximum to be expected in a chain with entirely immobilized anions.

We tried to explain this phenomenon by assuming that light increases the permeability of the responsible membranes to the comparatively small Cl′ ions by decreasing the electrostatic anion block (primary photoelectric effect), whereas the mobility of the more voluminous phosphate ions appears to be reduced within the illuminated diaphragm by the pore constriction because of the secondary photoelectric effect.

An application of this theory to our present problem seems to yield a satisfactory explanation for the behaviour of the living carrot tissue in the tested salt solutions: if the pieces are submerged in distilled water, then, owing to the natural electrolyte content of the tissue and its predominant cation permeability, the surrounding medium will become electropositive. An external solution of $N/10$ KCl imposes an inversely directed concentration gradient, which must cause the *tissue* to become electropositive in respect to the medium, without changing the original negative charge of the pore walls. Since, on the other hand, the suction force of the external solution is certainly less than that of the tissue, the resulting *electro-osmotic* component will be opposed to the *normal* osmotic suction of the cells.

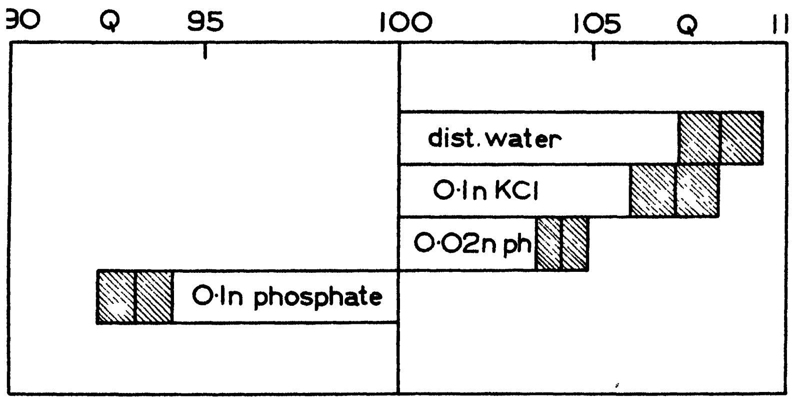

Fig. 8. Q values of the electrolyte series. Hatched field = single standard deviation of the mean.

Hence a reduction of this electro-osmotic force by light must become apparent as an increase of the water intake, as—for just the opposite reasons—in the experiments with distilled water, where the original potential difference rose under illumination.

In the phosphate mixtures, however, the situation is entirely changed. Here the diffusion potential caused by the *external* solution must be expected to be increased by light. This implies that the external medium will gain in negative charge, as the outward going diffusion of the cell electrolytes is reduced, i.e. as the external concentration is made greater. Thus the electro-osmotic component caused by the phosphate will be enhanced by light, partly or totally superseding the opposite electro-osmotic effect of the cell electrolytes. Since the former is again, as in the KCl experiments, opposed to the normal osmotic suction force of the tissue, this part of the light reaction must appear as a reduction of the total water intake.

In our diluted ($N/50$) phosphate solution the mechanism described only went far enough to reduce the "normal" light reaction from 108·3 to 104·2 (Q values), i.e. the main effect here seemed still to be controlled by the "natural" diffusion potential. In the $N/10$ solution, however, the direction of the light reaction appeared already completely determined by the superposed phosphate potential. The following diagram may help to explain our conception (Fig. 9). So the general result

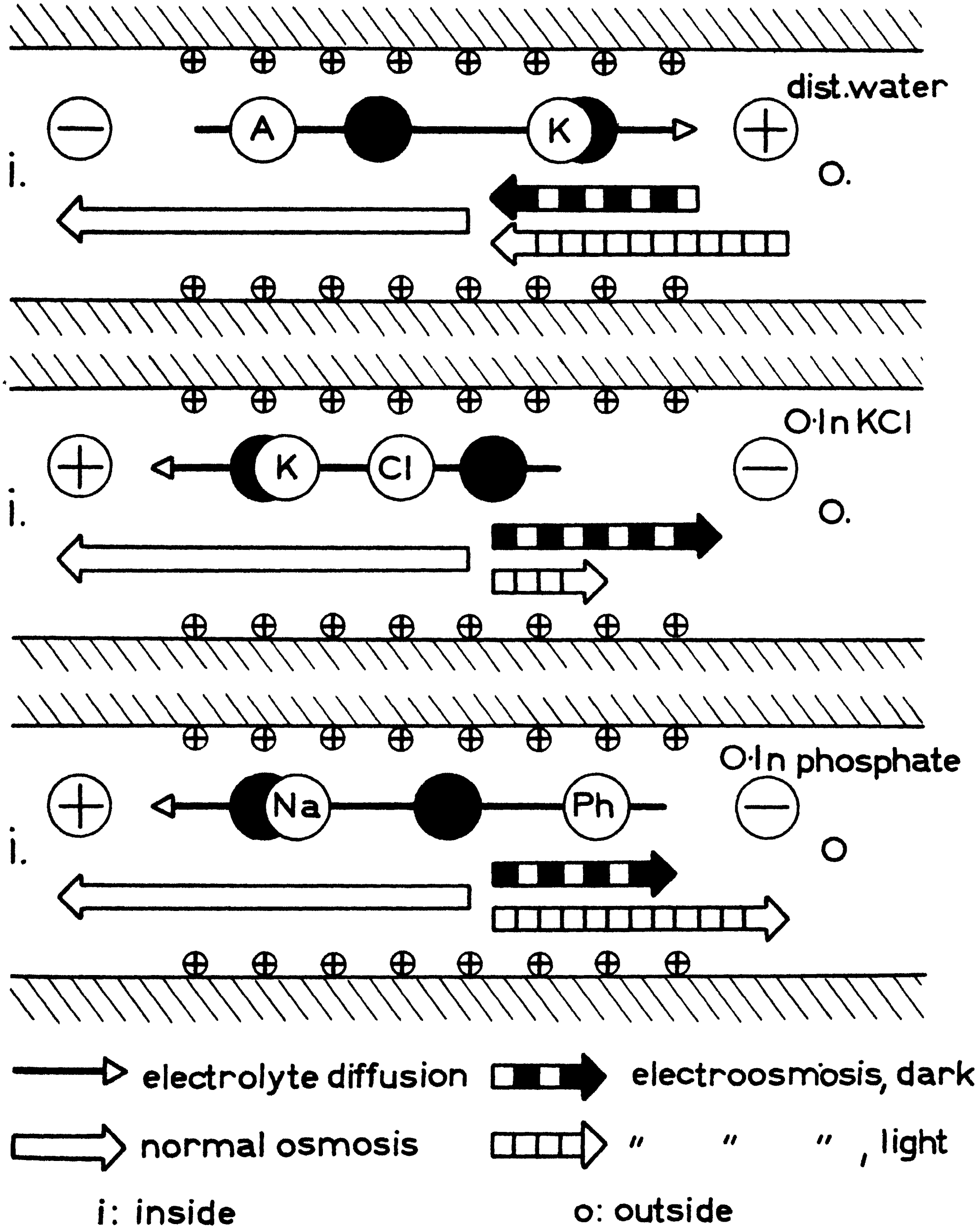

Fig. 9. Diagram of the interaction between normal and anomalous osmosis in light and dark. Each part represents one pore of the plasma membrane in contact with distilled water, $N/10$ KCl, and $N/10$ phosphate buffer respectively. ⊕ = positively charged boundary layer of water thread filling the pore. Ion-models indicate formation of the diffusion potential in darkness (●) and its modification by light (○). A = anion of the cell-electrolytes; Ph = anions of the phosphate mixture.

of our electrolyte-experiments seems to agree satisfactorily with the working hypothesis developed in the introductory chapter; as far as we are aware this conception explains our observations more consistently than the other views in question, and seems capable of putting the various phenomena concerned on a common basis.

VIII. SUPPLEMENTARY EXPERIMENTS ON THE SUCTION FORCE OF THE TISSUE AND THE SOLUTIONS USED; *p*H CONDITIONS

In order to complete our information about the physiological conditions in the reported experiments supplementary determinations were made of (*a*) the suction force of the fresh tissue at the beginning of the experiments, (*b*) the osmotic value of the used electrolyte solutions, and their isosmotic coefficients, and (*c*) the *p*H conditions in the various media.

The method employed throughout our suction force measurements was a gravimetric one resembling that suggested by E. C. D. Baptiste (1935). In spite of certain theoretically justified objections, concerning chiefly the possible intake of water and solutions by the intercellular system of the tissue, the method adopted yields surprisingly reliable results; as a detailed criticism of its principle would exceed the scope of the present paper it will be postponed to a later occasion. The technique followed was this: the tissue was cut into small cylinders as described above, well mixed and as uniformly distributed as possible into a number of small weighing glasses, usually ten pieces in each. Then the lots were weighed on an analytical balance to the third decimal place and immediately transferred in a sucrose concentration series tried out in previous tests; after 30 and 60 min. of immersion the samples were superficially dried, collected into their original weighing glasses and weighed again. The observed changes were calculated as percentages of the original weight. The exact suction force equivalent of the tissue, i.e. the sugar concentration in which neither gain nor loss of weight would occur, was graphically determined by plotting the percentage values against the corresponding concentrations. The intersecting point of the connecting curve with the abscissa indicated the desired concentration. Usually each set served two purposes: the determination of the suction force of the tissue and of the isosmotic coefficient of our two main electrolyte solutions, *N*/10 KCl and *N*/10 phosphate buffer. These latter values were found by the following simple procedure: since both solutions were hypotonic, the tissue absorbed water from them. By locating the measured percentage figures upon the simultaneously determined sugar graph, first the corresponding sucrose equivalent could be directly read from the abscissa in molarities, and then, if necessary, their equivalent pressure and atmospheres was computed from A. Ursprung's tables (1938).

Accordingly, each concentration series comprised the following eight steps: distilled water; 0·1, 0·2, 0·3, 0·4, 0·5 mol. sucrose; 0·1 *N* KCl; 0·1 *N* phosphate buffer. The sucrose solutions were prepared with pure crystalline sugar, the salt solutions with the same "purissimum" chemicals used in the previous "light" experiments. All sucrose concentration figures indicate volume molarities; salt

figures, respiratory normalities. The measurements were performed in daylight (north windows) at about 20° C. (Table 8).

Table 8. *Carrot tissue. Percentage change of original weight. Temp.* 20° C.; 30. iii.–13. iv. 1939

Exposure time min.	No. of experiments	H_2O	Vol. mol. sucrose 0·1	0·2	0·3	0·4	0·5	KCl 0·1 *N*	Phosphate 0·1 *N*
30	7	8·40	6·39	4·36	2·65	0·57	−2·24	+4·78	+5·38
60	5	9·89	6·70	4·54	2·56	0·63	−3·13	+5·13	+5·58

From the graphic representation of these figures (Figs. 10*a*, *b*) the corresponding suction force values, and isosmotic equivalents were extracted (Table 9).

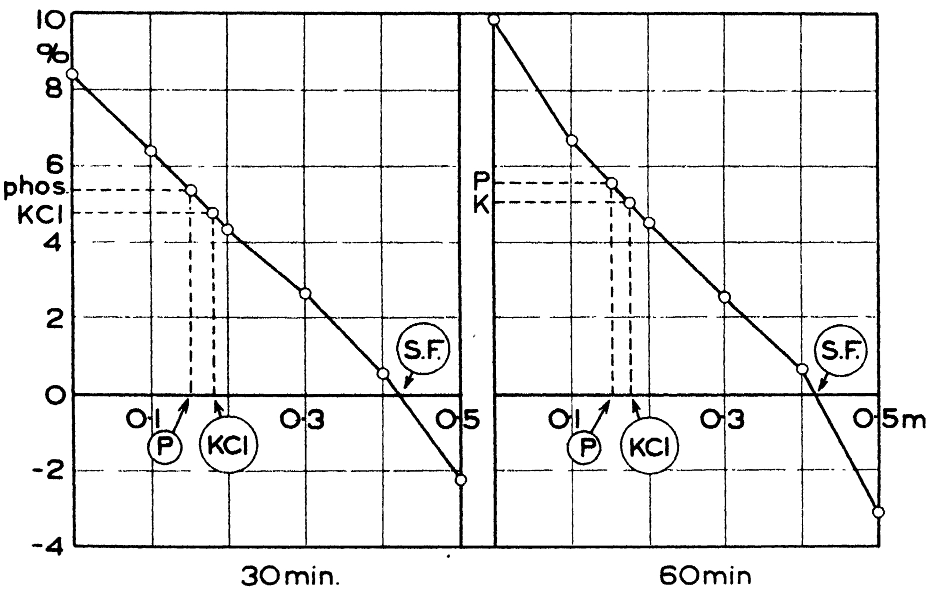

Fig. 10. Graphic determination of the suction force of the tissue (*S.F.*) and of the isosmotic coefficient of the 0·1 *N* KCl solution (KCl and K) and the 0·1 *N* phosphate buffer (phos. and *P*). Ordinate = percentage change in weight; abscissa = molar concentration of the external sugar solution.

Table 9

Exposure time min.	Suction force of tissue: Sucrose equiv.	Atm.	0·1 *N* KCl: Sucrose equiv.	Atm.	0·1 *N* phosphate: Sucrose equiv.	Atm.
30	0·425 ± 0·014	12·0	0·184 ± 0·004	4·92	0·152 ± 0·005	4·06
60	0·417 ± 0·020	11·7	0·176 ± 0·006	4·68	0·153 ± 0·007	4·09

Pressure values for 20·0° C.

These data reveal several interesting facts: first we notice that the suction force of the tissue shows a slight tendency to drop with time. Since this change occurs under static osmotic conditions we must attribute it to a loss of osmotic cell material by exosmosis, or more probably by respiration. Although the difference is small and does not exceed the standard deviation of the mean we still feel justified in accepting it as authentic, because it appears invariably in every co-ordinated set of 30 and 60 min. determinations performed with the same experimental material. The previously discussed shape of the water intake curve indicates, however, that this loss of suction force cannot last very long but must be followed by some kind of anatonosis.

Another striking fact is the surprisingly high sucrose equivalent of the $N/10$ KCl solution in our 30 min. experiments, which approaches the theoretical value as calculated from the corresponding degree of dissociation ($\sim 86\%$). Since the permeability of the tissue for this salt appears to be considerable (notice the drop of the isosmotic coefficient after 60 min.) there is reason to believe that the high initial activity of the solution must be attributed again to anomalous osmosis. We hope to return to this interesting question in a later paper.

The remarkably low isosmotic coefficient of our phosphate buffer is on the other hand very probably due to the poor electrolytic dissociation of the two salts concerned. The permeability of the tissue to this solution has to be excluded as a cause, since the coefficient observed after the first interval (30 min. value) remains practically constant during the next half hour. To what extent its value has been modified by an electro-osmotic component cannot be judged at present as reliable figures for the theoretical osmotic pressure of the employed mixture are not yet available.

We have finally to discuss in brief the pH conditions in our solutions which, according to W. Hertel's recent findings (1939) certainly influence the water permeability of the tissue. In a number of our "light" experiments we measured potentiometrically (quinhydrone electrode) the pH of our distilled water, and of all our salt solutions before and after the tissue was immersed in them for 30 min. (Table 10).

Table 10

Solution	H_2O	0·1 N KCl	0·02 N phosphate	0·1 N phosphate
pH before experiment	6·85	6·40	6·80	6·80
pH after 30 min.	6·60	6·20	6·80	6·80

Values reduced to 18° C. "Light" and "dark" samples identical.

The observed values show no considerable differences. KCl causes a slight acidification of its solution, though certainly not to such an extent as H. Drawert (1937) maintains, whose results must have been impaired by some impurity of his distilled water. In the unbuffered solutions the living tissue reduces the pH by 0·2–0·25 unit, probably by producing free carbonic acid, an effect which the phosphate mixtures seem to compensate completely. In no case, however, could

any influence of the illumination upon the acidity be discovered. After these experiences it seems rather improbable that the *p*H of the medium should have played a decisive role in the mechanism of the investigated photoreactions.

SUMMARY

1. The effect of incandescent white light upon the osmotic water intake and output by root tissue of *Daucus carota* has been investigated by a gravimetric method. The qualities of the light-field were: wave-length range 350–800 mμ; centre of gravity $\sim$700 mμ; intensity 36×10^{-3} cal./cm.^{2}/sec.

2. The temperature coefficient of the water intake of our material was found to be unexpectedly low. We observed the following Q_{10} values:

Range of temperature °C.	10–20	20–25	25–30
Q_{10}	1·1124	1·0800	1·1067

3. The photoreaction in distilled water was measured for times of exposure ranging from 5 to 120 min. It invariably appeared as an increase of the rate of water intake, the extent of which varied with the exposure time. If the magnitude of the reaction is expressed by the fraction $100 \times \frac{\text{water intake ``light''}}{\text{water intake ``dark''}}$, this quotient, Q, reaches a maximum of 108·3 after 30 min.

4. The water loss of the tissue in hypertonic sugar solutions (0·5 and 0·7 *M* glucose) is not affected by illumination. It has to be concluded that strong osmotic agents destroy the light sensitivity of the system.

5. Hypotonic electrolyte solutions affect the photoreaction of the water intake in a characteristic manner which is best explained by the assumption that the electric diffusion potential between tissue and medium plays a decisive role in the process. $N/10$ KCl reduces the effect slightly ($Q = 107·1$), $N/50$ sodium phosphate buffer considerably ($Q = 104·2$), whereas the same solution in higher concentration ($N/10$) reverses the direction of the reaction ($Q = 93·2$).

6. A comparison between these results and our previous experience with the photoelectric effect in organic membranes makes it very probable that the light-sensitive factor of the water intake is its electro-osmotic component. Following this conception, the character of the total reaction depends on two conditions: (*a*) the direction of the electro-osmotic component in relation to the normal osmotic suction-force, and (*b*) the sign of the photoelectric effect in the illuminated system.

7. In the last paragraph some figures are given concerning the osmotic suction-force of the tissue, the osmotic value of the solutions used and their actual acidity.

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ON THE "RESIDUAL" INTERPRETATION OF COASTAL FLORAS IN THE APPALACHIAN UPLANDS OF EASTERN UNITED STATES

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PRESENTING a discussion on coastal species in the Appalachian Uplands requires a survey of the work of Braun (1937*a*), Carr (1938*a*), Fernald (1931, 1937), Harshberger (1903), and Kearney (1900). In viewing the problem from a phytogeographical and ecological standpoint, these authors have sought to correlate the occurrence of coastal species in the uplands with the physiographic history of the province concerned. Such correlations combined with the disjunct ranges of the species have led to the residual interpretation. Geological evidence indicates that during late Cretaceous and early Tertiary the Appalachian Uplands were essentially reduced to a peneplane status, affording ideal conditions for a mesophytic plant growth. At this period the tropical groups were widespread in the North as shown by fossil record. Here the Coastal genera *Stenophyllus*, *Xyria*, *Eriocaulon*, *Sarracenia*, *Helonias*, and *Utricularia*, etc., prevailed along the low meandering streams and in the swampy areas, until an uplift of the Schooley peneplane (Cretaceous) altered the conditions, in turn initiating a recession of the mesophytic and hydrophytic types to the available coastal strip. Mesophytes gave way to the developing xerophytes. However, relics of this flora still persist in segregated colonies in the Appalachian Uplands where ponds, bogs, and grassy meadows prevail.

Braun (1937*a*) reports such relics as *Uniola laxa* (L.) B.S.P., *Panicum longifolium* Torr, *Cleistes divaricata* (L.) R.Br., *Bartonia paniculata* (Michx.) Robinson, *Lobelia Nuttallii* R. & S., and *Orontium aquaticum* L. from the Cumberland Plateau in Kentucky. All of these plants are to-day primarily confined to a narrow strip along the Atlantic Coast of Eastern United States, and show marked affinity with tropical groups. Of great interest is the report of *Cleistes* from the standpoint of extreme antiquity. To-day it shows a local distribution in the uplands, and has retreated only slightly to the Coastal Plain. Fernald (1937) comments on its slight occurrence in south-eastern Virginia in a region contributing such local plants as *Xyris Curtissi* Malme, *Burmannia biflora* L., and *Utricularia virgatula* Barnhart. Its extreme antiquity is indicated by its scattered and disjunct range. It shares equal rank on the relic list with *Helonias bullata* L. which holds a similar, local distribution with slight intrusion in the coastal region.

Following Fenneman (1928), Braun concludes that the Cumberland peneplane represents only the eroded and reduced Schooley peneplane (Cretaceous). Braun (1937*a*) writes: "The present known distribution of most of the coastal plain plants in the Cumberland Plateau coincides with the undissected remnants of the Schooley

or Cumberland peneplane." This consideration leads her to assert that the occurrence of the coastal species on undissected remnants of the plateau or on monadnocks and their wide separation from the general area of their ranges point to the relic interpretation.

Considering the floral aspects of the Augusta County bogs in south-eastern Augusta County, Virginia, the author (1938*a*) presents a striking list of distinct and characteristic coastal species from this region. He finds growing on the west side of the Blue Ridge at an altitude of approximately 1600 feet in bogs, ponds, and meadows *Lygodium palmatum* Bernh. Sw., *Rynchospora gracilenta* Gray, *Stenophyllus capillaris* (L.) Britton, *Scleria reticularis* var. *pubescens* Britton, *Xyris caroliniana* Walt., *X. flexuosa* Muhl., *Sabatia gracilis* (Michx.) Salisb., *Eriocaulon septangulare* With., *Rhexia mariana* L., *Solidago tenuifolia* Pursh., *S. graminifolia* var. *platyphylla* Fernald, etc., and five species of *Utricularia*, *clandestina* Nutt., *gibba* L., *sublata* L., *radiata* Small, and *fibrosa* Walt. *Hibiscus palustris* L. and *Lysimachia radicans* Hook. are of noteworthy occurrence here. These plants holding such a marked affinity with tropical groups accompanied by their disjunct range and occurring with such established relics as *Helonias bullata*, *Orontium aquaticum* L., Ell., *Galax aphylla* L., *Trillium pusillum* (Peck) Nash, etc., with a similar affinity, may all be placed in the category of relics that were present on the Valley (Shenandoah) and in the lower flatwoods of the Blue Ridge during peneplanation, and persisted there during the plant recession following uplift.

For the physiographic history of the region considered in the author's (1938*a*) report, Watson & Cline (1913) may be cited. It is indicated in their work that the region now occupied by the Shenandoah Valley has been subject to erosion since the close of the Paleozoic era. Four distinct cycles of erosion (peneplanation) are defined.

In the Kittatiny Cycle the entire region was reduced to a peneplane except in hard stone areas in the Alleghany Ridge and the Blue Ridge. The cycle was completed in Cretaceous time. An uplift of the Kittatiny Plain rejuvenated the streams, and a second cycle of erosion was inaugurated in early Tertiary. At the close of the Tertiary cycle streams were rejuvenated by uplift of the region, and a new cycle designated as the Shenandoah was begun which resulted in the development of the Shenandoah plain in the valley limestone. A third uplift of the region brought the Shenandoah cycle of erosion to a close, and thus was entered upon a new or recent cycle of erosion in early Pleistocene. From this historical sketch it appears that the physiographic features of the region have been favourable for the maintenance of the now recognized coastal types that were once spread as a cosmopolitan flora during Cretaceous and Tertiary times. A similarity of physiographic features is noted in other upland regions where coastal species occur.

Harshberger (1903) and Kearney (1900), commenting on coastal plants in the mountains of North Carolina and Tennessee, have expressed the opinion that the progenitors of the austro-riparian plants of the mountains and coastal plain, specifically identical, mingled when the country was a level peneplane, becoming different as the elevation of the land became more marked.

Fernald (1931), considering the general problem of plant segregation, presents

the disjunct ranges of certain families abundantly represented by coastal species, some of which I have discovered in the Augusta County bogs of Virginia. The Haemodoraceae as mapped by Fernald shows a sharp tropical distribution appearing for the most part in Australia, the southern tip of Africa, Central America, and northern South America. From Augusta County I report a representative of this family, *Lachnanthes tinctoria* (Walt.) Ell. This report is of striking interest as it represents the only known inland station for this extremely coastal and tropical group. At once its disjunct range is noted, and logical explanation for its occurrence is toward the residual interpretation. Representatives from the Schizaeaceae, Xyridaceae, Lentibulariaceae, Araceae, Liliaceae, and Magnoliaceae, etc., holding a similar tropical range, are present in the uplands, being especially conspicuous in an extremely small area in south-eastern Augusta County.

It appears significant to express Professor Fernald's opinion here as it relates to the residual idea in general. Speaking of coastal species in the uplands, he writes: "These plants are of peculiar interest because, occurring on the old and now elevated core of eastern North America, they seem to be true relics of the Cretaceous or early Tertiary flora which, upon the elevation of the old Cretaceous peneplane from its sea-level status, partially moved outward to the newly available Coastal Plain. This general situation has been discussed or outlined several times and more and more the evidence accumulates that many species now characteristic of the Coastal Plain were formerly on the ancient core of eastern North America, in our latitude the Appalachian and Ozarkian Uplands. Some students maintain that Coastal Plain species are moving into the ancient uplands. They may be right in this interpretation. In general, however, it seems to me more probable that plants and animals of long established and conservative groups should have moved out from the ancient lands during pronounced changes due to uplift and have entered the newer or younger areas as they became available for occupation, than that groups already conservative should have arisen upon the very young areas and then have intruded themselves successfully into the old regions where ancient series of species were already in possession."

Summary

1. In certain bogs, ponds and grassy meadows of the Appalachian Uplands, eastern United States, characteristic coastal species have been noted. In the Augusta County bogs, in Virginia, this flora is especially conspicuous and pronounced.

2. From a phytogeographical and ecological standpoint Braun, Carr, Fernald, Harshberger, and Kearney have discussed the erratic and disjunct distribution of the coastal plants.

3. Correlation of the physiographic history of the provinces concerned, combined with the disjunct ranges of the species has led to the residual interpretation.

I express my appreciation to Dr Ivey F. Lewis, University of Virginia, for his sustained interest and advice.

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THE CARBOHYDRATE METABOLISM OF GERMINATING BARLEY

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(With 3 figures in the text)

Introduction

The following account of the carbohydrate metabolism constitutes one phase of an investigation into the respiration of germinating barley grains which is being carried on in the Plant Physiological Laboratories at Oxford, and in order that results from different phases of the work should be directly comparable the various observations have been made on a pure line of barley, variety Plumage Archer, grown under standard environmental conditions. The analyses described in this paper were all carried out on grain of the 1934 harvest.

The grains were germinated in darkened plant chambers of the type described by James & Norval (1938), at a temperature of 21° C., on sand moistened with a culture solution containing all the essential mineral elements and known to give good results with barley (James, 1931). At the end of the allotted period of germination the seedlings were detached from their endosperms, cut up, and plunged into boiling alcohol containing a little sodium bicarbonate to neutralize any acids present. The sugars were extracted by diluting the alcohol to 80 % and boiling for 6 hr., fresh alcohol being substituted at the end of the second and fourth hours. No additional sugar was obtained by further boiling. The three extracts were mixed and the alcohol distilled off under reduced pressure at a temperature of approximately 35° C., the residue then being redissolved in warm water. The aqueous solution so obtained had a golden brown colour due to the presence of a pigment, apparently of the anthoxanthin type, and no method was discovered by which it could be entirely removed without affecting the sugar content of the solution. The presence of a small amount of the pigment did not interfere with the final titrations since these were carried out in an acid medium when the colour is only very slight and the pigment itself has no reducing power. The method finally adopted for clearing the solution of colloidal matter and protein consisted of centrifuging out added aluminium hydroxide, which removed most of the pigment although the solution still retained a definite colour when alkaline. That the application of this method of clearing does not lead to appreciable loss of maltose, sucrose, glucose or fructose, was shown by the following test. A solution was prepared containing approximately 0·2 % sucrose and 0·1 % maltose. Two 20 c.c. samples of this solution were taken, one being cleared and made up to 50 c.c., and the other diluted to 50 c.c. with distilled water. The sample to be cleared was transferred to a centrifuge tube, 2 c.c. of a 20 % suspension of aluminium hydroxide added, and allowed

to stand for 10 min. with occasional shaking. It was then centrifuged for 15 min. and the clear supernatant fluid decanted off. The residue in the tube was washed with warm distilled water and again centrifuged. Two such washings were found sufficient to ensure that no sugar remained in the tube.

The amount of maltose present in 5 c.c. aliquots of each sample was determined by means of the Hagedorn-Jensen titration. The sucrose present was estimated by comparing the reducing power of the solution before and after mild acid hydrolysis. After hydrolysis the solution contained maltose and invert sugar, and a sample was cleared and the reducing power again estimated. The results of the titrations are recorded in Table 1.

Table 1. *Results of titrations in c.c.* 0·01 *thiosulphate per* 5 *c.c. aliquot*

(*a*)	Initial reducing power of the solution (maltose)	3·69
	R.P. of the solution after clearing	3·69
(*b*)	R.P. after inversion of the sucrose	8·28
	R.P. of sample cleared prior to inversion of sucrose	8·27
(*c*)	R.P. of sample cleared after inversion of sucrose	8·24

In section (*a*) of this test the effect of the clearing technique on the maltose component of the solution is observed, while any loss of sucrose would be revealed in section (*b*). Section (*c*), taken in conjunction with section (*b*), shows the effect of clearing a solution containing maltose and invert sugar. In no case is the loss of reducing power in the course of manipulation as much as 1 %.

THE ESTIMATION OF REDUCING POWERS

In all the estimations, except those of maltose and hexose, Hanes's (1929) modification of the Hagedorn-Jensen titration was used in conjunction with Widdowson's (1931) calibration figures. For the estimation of maltose the iodimetric method is more suitable since hydrolysis of maltose to glucose results in an increase of nearly 100 % with this titration whereas the Hagedorn-Jensen titration shows only 25 % increase (Lehmann, 1931; Yemm, 1935). Yemm's modification of Widdowson's technique was therefore used in these analyses.

METHODS USED FOR ESTIMATING INDIVIDUAL CARBOHYDRATE FRACTIONS

Sucrose was estimated by observing the increase in reducing power on incubating an aliquot of the extract with invertase. The titration values were converted to milligrams of invert sugar using Widdowson's figures, and the weight of sucrose originally present calculated from the equation

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6.$$

When raffinose was also present an appropriate deduction was made to allow for inversion of the sucrose component during incubation. The enzyme used was tested on a solution of sucrose and on a solution containing both sucrose and maltose, and the results obtained compared with the increase observed on mild acid hydrolysis of the sucrose solution. The titration values obtained in this test are recorded in Table 2. It is evident from these results that the presence of maltose

does not affect the accuracy of the estimation, and the values obtained by enzyme hydrolysis were within 1 % of that obtained with acid hydrolysis.

Table 2. *Test of invertase preparation used in the estimation of sucrose. The figures represent c.c. thiosulphate per 5 c.c. aliquot of sugar solution. Hagedorn-Jensen titration*

	Before hydrolysis	After hydrolysis by invertase	R.P. due to invert sugar
1. Sucrose solution	Nil	7·40	7·40
2. Sucrose + maltose	1·84	9·26	7·42

The reducing power of the sucrose solution after mild acid hydrolysis was 7·47.

Maltose was estimated by observing the increase in reducing power with diastase, as measured by the iodine-oxidation titration. Part of the increase being due to glucose derived from the inversion of sucrose, the increase registered by this titration on incubation with invertase was also noted and deducted from the value obtained with the diastase digest. The procedure followed was that described by Yemm (1935).

The presence of the trisaccharide raffinose in some seedlings having been reported by Colin & Belval (1934), a method was developed by means of which this sugar could be detected and estimated in the extracts. In a pure solution the estimation is not difficult, but when sucrose is also present the position is complicated by the similarity between the reactions of the two sugars to hydrolysis with either invertase or mild acid. By either of these methods the fructose component of raffinose is split off, leaving the glucose and galactose in the form of melibiose, a very stable disaccharide having a reducing power approximately equal to that of glucose. Raffinose itself has no initial reducing power, so that the usual methods of estimating sucrose fail to distinguish between the two sugars. Raffinose is usually estimated polarimetrically but this method is not suitable for the small quantities and low concentrations encountered in my extracts. An attempt was therefore made to utilize the action of the α-galactosidase in emulsin, which splits off the galactose leaving the glucose and fructose combined in the form of sucrose. This reaction suffers from the disadvantage that it takes a week or longer to reach completion, but as a result of preliminary experiments it was found that when the enzyme is present in excess and the *p*H of the digest is carefully adjusted to 5·4, there is a direct relation between the amount of raffinose present and the increase in reducing power of the digest after 24 hr. incubation at 30° C. Four digests were prepared containing 2, 4, 8, and 10 mg. raffinose respectively. Each also contained 5 c.c. of a 0·5 % solution of emulsin, 5 c.c. of an acetate buffer solution *p*H 5·4, and sufficient water to make the volume up to 25 c.c. A few drops of toluol were added to ensure sterility and the digests were incubated at 30° C. for 24 hr. The reducing power of aliquots withdrawn before and after incubation was determined by means of the Hagedorn-Jensen titration. The increase obtained in each case is recorded in Table 3, and in Fig. 1 the results are expressed graphically, milligrams of

raffinose being plotted against the corresponding decrease in the number of c.c. of thiosulphate required for the titration after incubation.

Table 3. *Estimation of raffinose*

Mg. raffinose in the digest	Increase in R.P. of the digest after 24 hr. incubation. c.c. thiosulphate
2	1·44
4	2·12
8	4·24
10	5·36

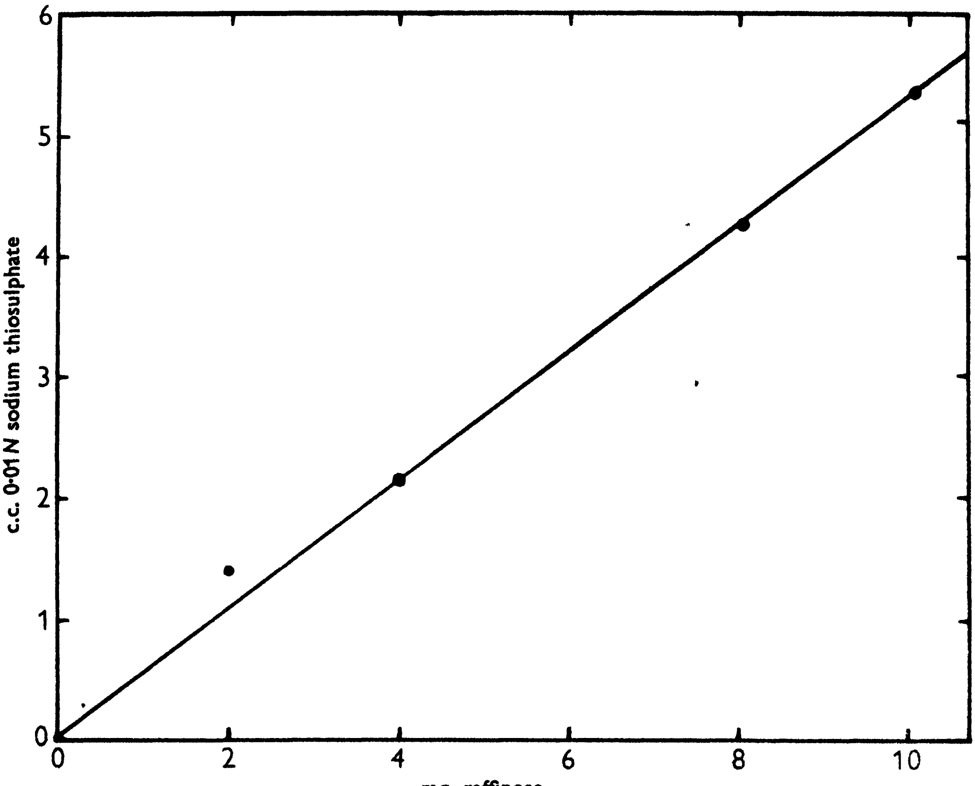

Fig. 1. Increase of reducing power after 24 hr. emulsin hydrolysis of raffinose at 30° C., pH 5·4. Hagedorn-Jensen titration.

Each of the sugar extracts obtained from the seedlings was submitted to emulsin hydrolysis in this way. The enzyme used was shown to have no effect on either maltose or sucrose by incubating it with a solution containing both these sugars. There was no increase in reducing power after 24 hr. incubation at 30° C.

It was originally intended to estimate the hexose sugars from the loss of reducing power on fermentation with yeast, but the results obtained in this way were unsatisfactory owing to the production of interfering substances of high reducing

power during the course of fermentation. A method was therefore developed for estimating these sugars from the carbon dioxide production during fermentation (James & James, 1936). Before fermentation the extract was incubated with diastase to hydrolyse the maltose and sucrose present, and the hexose equivalent of these sugars was deducted from the observed value. The figures for hexose originally present obtained in this way have a rather wide margin of error, since they may be affected by the accumulated errors of the maltose, sucrose and raffinose determinations. The method of estimation provides a figure for total hexose after hydrolysis with an error of probably not more than 3 %, but the estimation of sucrose and maltose may be equally in error. The cumulative error may therefore be considerable.

The carbohydrate components of the insoluble residue remaining after the sugars had been extracted from the seedlings were estimated in two fractions. It is difficult to distinguish between the various components of the cell walls and to separate cellulose from hemicellulose, and the only division attempted in these experiments was an arbitrary one based on degree of resistance to acid hydrolysis. The first of the two fractions consisted of those substances broken down by mild hydrolysis and includes at any rate the greater part of the hemicelluloses and also the small amount of starch present. The second fraction, comprising substances broken down by a subsequent stronger hydrolysis, includes true cellulose and possibly also some of the more resistant hemicelluloses.

Upon completion of the alcohol extraction the residue of the seedlings was washed, dried in a vacuum desiccator at 50° C., and ground to a powder. For the determination of the first of the two fractions this powder was submitted to mild hydrolysis by boiling for 2 hr. with 3 % sulphuric acid. The acid was then filtered off through asbestos, to avoid the use of filter paper, neutralized, and the reducing power of the solution determined by means of the Hagedorn-Jensen titration. For the second fraction the residue from the first hydrolysis, together with the asbestos filter, was washed and again dried. The dried residue was then subjected to strong hydrolysis with 72 % sulphuric acid for 3 hr. at room temperature, after which the acid was diluted to 3 % and a second mild hydrolysis carried out as before. The reducing power of the solution so obtained was estimated and the values of both fractions expressed as milligrams of equivalent hexose.

Results of the analyses

Two series of analyses were made, one on excised embryos germinated in the plant chambers on sand moistened with a culture solution containing all the essential mineral elements but no carbohydrate, and one on embryos from entire grains germinated under the same conditions. Successive batches of seedlings were analysed at different stages of the life history.

For the first series the grains were depaleated and kept for 2 hr. on moist sand at 21° C. The embryos were then excised and placed in the plant chamber with the scutellum in contact with the sand moistened with culture solution. The samples were divided into batches and germinated in separate chambers so that the time

occupied by the manipulations should cause as short a break as possible in the continuity of the conditions of germination, which was considered to start at the moment when the depaleated grains were placed on the moist sand. At the end of the allotted period of germination the chambers were opened in sequence and the seedlings cut up and plunged into boiling alcohol. The first analysis was carried out on a sample consisting of 2000 embryos after 2 hr. germination. Subsequent analyses were made on samples germinated for 24, 48, 96, and 144 hr. respectively. The results of the estimations are contained in Table 4 and Fig. 2.

Table 4. *Analyses of seedlings from excised embryos. The results are expressed as milligrams of equivalent hexose per* 100 *embryos in each case*

Hours germination	Sugars				Insoluble carbohydrate	
	Sucrose	Maltose	Raffinose	Maximum hexose	"Cellulose" fraction	"Hemi-cellulose" fraction
2	15·26	0·58	5·86	1·04	2·03	19·63
24	0·07	1·86	1·02	1·05	4·44	23·32
48	0·26	0·66	1·04	1·61	3·52	22·70
96	0·00	0·73	0·72	2·32	2·68	19·75
144	0·13	0·50	0·50	2·72	2·66	13·92

For the second series of analyses batches of entire grains were germinated in the plant chambers. At the end of the period of germination the seedlings were removed from their endosperms, washed thoroughly, and then cut up and plunged into boiling alcohol as before. Starting with seedlings after 2 hr. in the plant chamber a series of analyses was made on samples germinated for periods up to 14 days. The results of the estimations are shown in Table 5 and Fig. 3.

Table 5. *Analyses of seedlings from entire grains. The results are expressed as milligrams of equivalent hexose per* 100 *seedlings in each case*

Days germination	Sugars				Insoluble carbohydrates	
	Sucrose	Maltose	Raffinose	Hexose	"Cellulose" fraction	"Hemi-cellulose" fraction
0	15·3	0·58	5·86	1·04	2·30	19·6
1	5·4	—	0·00	—	3·48	22·8
2	0·0	9·33	0·00	2·40	4·92	35·8
3	31·0	4·60	0·00	2·50	5·80	46·8
5	60·0	11·97	0·00	1·04	16·08	91·2
6	58·5	—	0·00	—	22·44	104·4
7	29·0	20·85	0·00	6·52	23·52	177·6
8	24·9	—	—	—	—	243·0
9	39·0	—	—	—	—	282·0
10	33·6	22·00	—	13·15	22·92	266·0
12	10·5	24·03	—	14·02	—	—
14	2·8	2·33	—	10·99	—	—

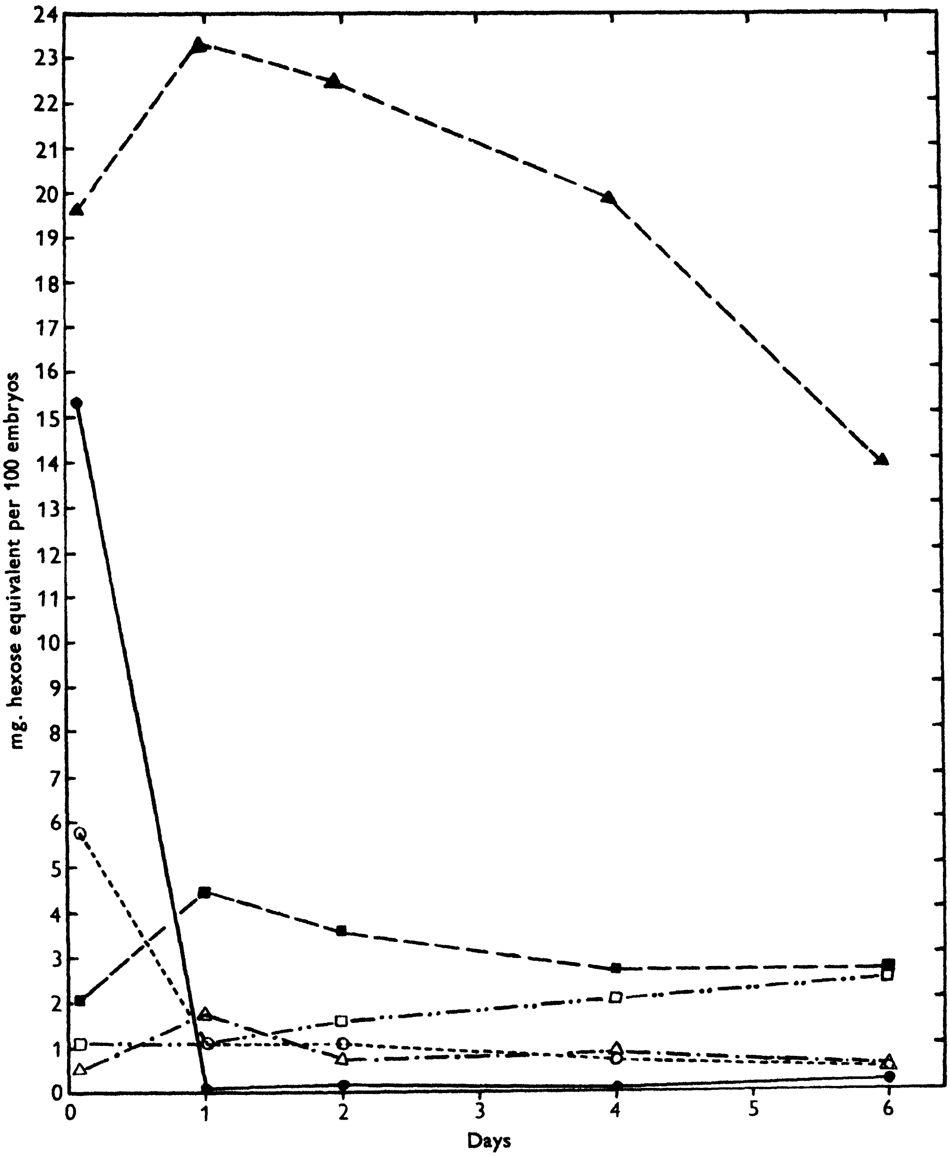

Fig. 2. Analyses of seedlings from excised embryos germinated on a culture solution containing no carbohydrate.

——●—— sucrose. ----○---- raffinose. —·—△—·— maltose. —··—□—··— maximum hexose. — — ■ — — "cellulose" fraction. — — ▲ — — "hemicellulose" fraction.

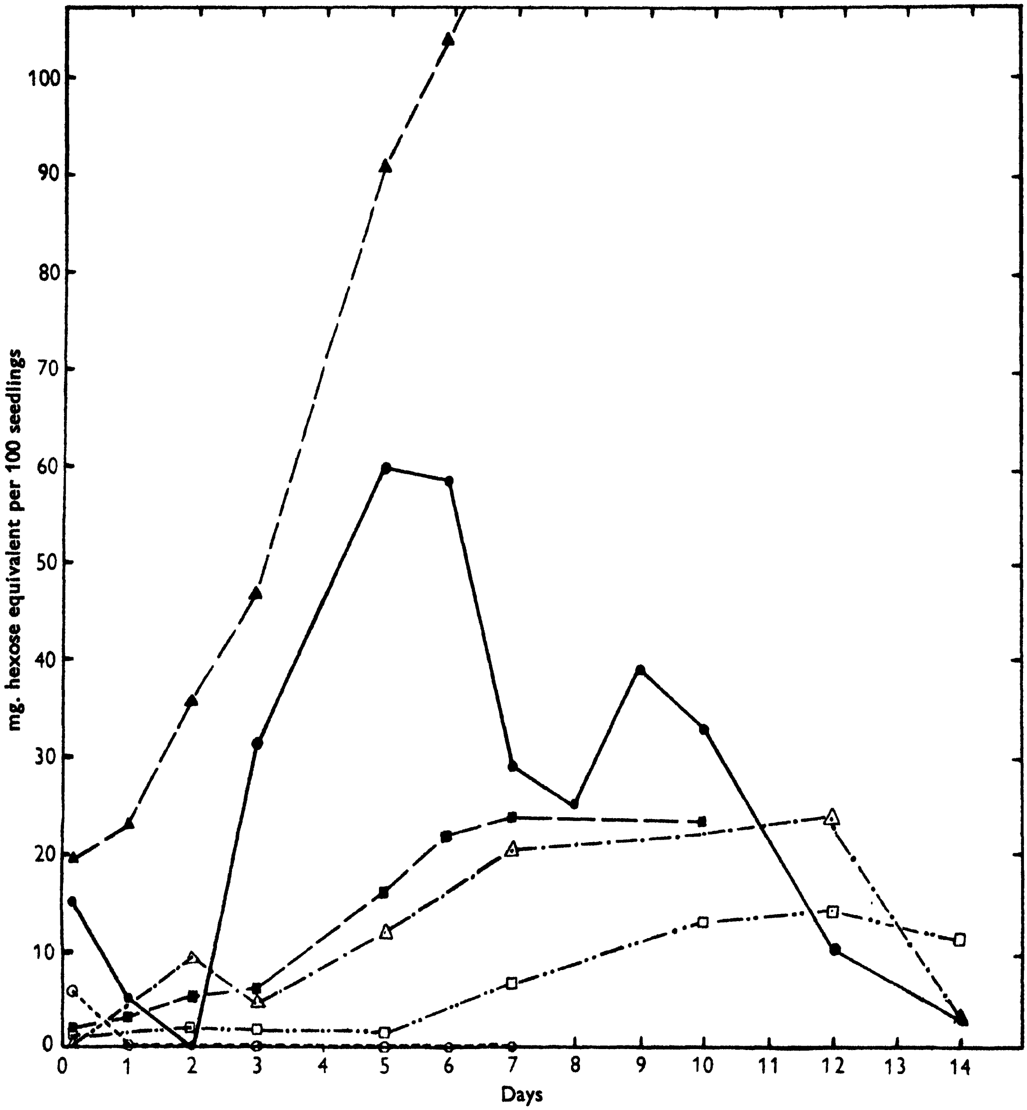

Fig. 3. Analyses of seedlings from entire grains.

——●—— sucrose. - •–△–• - maltose. — — ■ — — "cellulose" fraction.
- - - - ○ - - - - raffinose. –••–□–•• – hexose. — — ▲ — — "hemicellulose" fraction.

DISCUSSION

In considering the results of the two series of analyses and the conclusions to be drawn from them, it is necessary to keep in mind the fact that the seedlings were grown in the dark. This has little or no effect on the very early stages of the life history since at that time the materials for growth and respiration are available in excess from the endospermic reserves; but as soon as the rate of supply from these

reserves becomes less than that required to provide for the needs of the seedling, then the absence of further production by photosynthesis leads to starvation conditions of progressively increasing severity.

The first analysis, made 2 hr. after the grains were brought into contact with water, shows the position in the embryo at the commencement of germination. The most abundant individual sugar is sucrose. Reducing the amounts of each sugar to the common basis of hexose equivalent, sucrose provides 66 % of the total, the remaining fraction being made up of raffinose 25·8 %, maltose 2·6 %, and hexose 5·2 %. The hexose equivalent of the insoluble carbohydrates is approximately equal to that of the sugars. There is comparatively little true cellulose present, the "Hemicellulose" fraction representing 88 % of the total.

With the acceleration of germination there is a rapid change both in the actual amounts and the relative proportions of the individual carbohydrates. For the first 24 hr. the changes consist of a rearrangement of material already present in the embryo, coupled with a loss of such sugar as is utilized in respiration. The entry of sugar from the endosperm is evidently very slow, at any rate during the greater part of this period, for it exercises very little influence on the carbohydrate metabolism. This is shown by a comparison between the changes which take place in embryos germinated on their endosperms and those which are excised and grown on sand moistened with a culture solution containing no carbohydrate (cf. Figs. 2, 3). The production of fresh cell wall material indicated by the increase in the insoluble fractions is as great in the excised embryos as in the germinating grains, both show a slight accumulation of maltose and in neither is there any marked accumulation of hexose. In both cases there is a rapid loss of sucrose and raffinose, which also contains a sucrose unit, and only here is there any clear indication of the entry of sugar derived from the reserves in the endosperm. Sucrose almost entirely disappears from the excised embryos, whereas those germinating on their endosperms still contain approximately 33 % of the amount present after 2 hr. It will be seen that during the first 24 hr. the greater part of the sugar in the embryo has either been lost in respiration or used in the production of insoluble material. In the germinating grains sugar is now entering the embryo sufficiently rapidly for these processes to be maintained at an even higher rate than before, but in the excised embryos, which have no such source of supply, lack of sugar soon becomes evident in the metabolic changes which ensue. During the second day there is an actual loss of insoluble material both in the "Cellulose" and "Hemicellulose" fractions, in contrast to the embryos of entire grains in which both these fractions show an appreciable increase. There is also a slight fall in the amount of maltose present, but small amounts of this sugar remain throughout the period examined. Degradation of components of the "Hemicellulose" fraction continues with increasing rapidity for the remainder of the period of the experiment and by the end of the sixth day this fraction has fallen by 9·40 mg. per hundred embryos, a loss of 40 % of the amount present after 24 hr. germination. The "cellulose" fraction also decreases in the same proportion, 1·78 mg., representing 40 % of the total, being lost between the ends of the first and sixth days' germination. Thus

lack of a supply of sugar brings about a reversal of the normal course in the embryo at this stage. Instead of fresh cell wall material being deposited, some of that already present is broken down to provide a substrate for respiration. Qualitative tests after 24 hr. germination showed that in spite of the rapid loss of sugar a little starch had appeared in the root cap and at the base of the plumule and coleoptile, although none was present when the embryo was excised. This gradually decreases in amount and finally disappears, though traces remain in the root cap and at the base of the coleoptile until the fifth day. By the end of the first 24 hr. all the sugars had reached a low level, and none of those estimated show any subsequent increase. The figure for maximum hexose rises slowly but never becomes very high and in view of the degradation processes taking place this figure is probably in excess of the actual value, especially in the later stages. All evidence of respiration in these starved seedlings comes to an end after about 10 days.

In the seedlings germinated from entire grains, carbohydrate starvation is deferred by the entry of sugar from the endosperm, but, since this source of supply is limited and no further synthesis is possible while the plants are kept in the dark, a position similar to that in the excised embryos is eventually reached. For the first few days the rate at which sugar enters the embryo increases rapidly and the processes associated with germination and growth of the seedling show a corresponding acceleration. During the second day there is active production of new cell walls at the growing points. Both "Cellulose" and "Hemicellulose" fractions increase, the latter by nearly four times as much as during the first 24 hr., and an increasing amount of sugar is being used as a substrate for respiration. The rate of supply, however, is still not equal to the rate of loss, for sucrose continues to decline and at the end of the second day has disappeared entirely from the embryo. During the following 24 hr. the rate of entry increases sufficiently for sucrose to accumulate in spite of the rising respiration rate and the continued production of cell wall material, maltose alone declining. During the fourth and fifth days there is a general increase both of cell wall material and sugars, only the figure for hexose recording a small decline which is not comparable with the gains shown by the other fractions. By the end of the fifth day, however, the rate of supply is beginning to fall off. Synthesis of cell wall material is maintained but there is a decline in the amount of sucrose present, which becomes even more pronounced during the seventh day. Sugar is still entering the embryo but a greater amount is being used and the carbohydrate metabolism during the sixth and seventh days is similar to that of the early stages before the rate of entry of sugar from the endosperm had become sufficiently great to meet the demands made upon it. Sucrose alone decreases, the other sugars and the insoluble fractions continuing to increase. The fall in sucrose content is temporarily checked during the ninth day, when it again accumulates, possibly owing to the degradation of starch which had previously been synthesized when the supply of sugar was more abundant. At the same time the rate of production of "Hemicellulose" becomes slower, there is no appreciable accumulation of cellulose or maltose and the respiration rate declines. After the ninth day definite lack of sugar becomes evident, with results similar to those observed in the

excised embryos, sucrose and "hemicellulose" being the first to show a pronounced decline.

It becomes clear from this examination of the curves that the sucrose content of the embryo varies much more widely and rapidly than that of any other individual sugar. When a plentiful supply of carbohydrate is available it is the most abundant, while under starvation conditions it soon falls to a level below that of the others. The relation between the rate of entry of sugar from the endosperm and utilization in the embryo is most clearly and rapidly reflected in the curve showing the amount of sucrose present. In the initial stages, before the supply of sugar has become adequate to meet the loss in respiration and synthesis, sucrose and raffinose alone decline, while in the later stages, when the rate of entry of sugar becomes slower owing to depletion of reserves in the endosperm, sucrose again declines before the other sugars, no raffinose being present. During the period when the rate of entry of sugar is well in excess of the loss, sucrose accumulates to a much greater extent than maltose or hexose. From the rapidity with which these marked fluctuations occur, it seems that sucrose both appears and is consumed more rapidly than the other individual sugars. While these experiments do not afford direct evidence as to the form in which sugar enters the embryo from the endosperm, they afford an indication that a large proportion of it is likely to be in the form of sucrose.

Raffinose appears to occupy a similar position to sucrose in the provision of a substrate for metabolic processes, the addition of the galactose unit having no apparent effect on the availability of the sucrose component. It is present in the ungerminated grain but quickly disappears and there is no evidence that further synthesis takes place once germination has commenced.

Summary

1. The methods used for estimating individual carbohydrate fractions are described, including a method for the detection and estimation of small quantities of raffinose in the presence of sucrose.

2. Fluctuations in the amounts and relative proportions of the various fractions during germination and starvation are followed by means of two series of analyses in which the individual carbohydrates are estimated at successive stages of the life history under definite environmental conditions. In the first series excised embryos were germinated on sand moistened with a culture solution containing all the essential mineral elements but no carbohydrates, and in the second series entire grains were germinated under the same conditions.

3. The results of the analyses are discussed and it is shown that changes in the trend of carbohydrate metabolism are more clearly and rapidly reflected in the content of sucrose than that of any other individual sugar.

4. It is suggested that sugar from the reserves in the endosperm enter the embryo largely in the form of sucrose and to a lesser extent in the form of maltose.

5. The role of sucrose in the metabolism of the seedling is discussed and the conclusion reached that when an adequate supply is available sucrose provides the greater part of the substrate for respiration and synthesis of cell wall material,

maltose, etc. only being utilized to a comparable extent when the sucrose content has fallen to a low level.

6. Raffinose appears to provide a substrate as readily as sucrose. It is present in the ungerminated grain but there is no indication from these experiments that it is synthesized after germination has commenced.

It is a pleasure to express my thanks to Dr W. O. James for helpful suggestions and discussion throughout the course of this work.

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THE RESPIRATION OF BARLEY GERMINATING IN THE DARK

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(With Plate 3 and 16 figures in the text)

THE experiments reported in this paper have all been carried out with barley grains, var. Plumage Archer, supplied annually by Messrs Sutton and Sons. The aim was to investigate the drift of carbon dioxide emission and oxygen absorption resulting from the breakdown of the grains' reserves. They were, therefore, kept continuously in the dark from the moment of first coming into contact with water until death occurred. A constant temperature of 21° C. and the normal atmospheric gas mixture were employed throughout. In each experiment grain of the most recent harvest was used.

METHODS OF GAS ANALYSIS

Two principles have been employed: the precipitation of carbon dioxide by baryta in Pettenkofer tubes, and the "constant pressure" analysis of carbon dioxide and oxygen (Haldane).

These familiar methods were chosen because they are already well tested in respiration work and because of their simplicity. They give consistent and readily comparable results for carbon-dioxide emission and do not show personal errors when used by different members of a team. They can also both be adapted to the analysis of a continuous series of samples at frequent intervals; our series have sometimes lasted for several weeks. To fulfil all requirements two variants of the Pettenkofer method have been found necessary (App. 1, 2) and two variants of the Haldane (App. 3, 4).

Apparatus 1

In this apparatus a stream of air led in from out of doors is scrubbed free from carbon dioxide by a soda lime tower and a wash bottle containing baryta water. The air is then led into a coil or bubbled through water in a thin-walled chamber immersed in a thermostat bath at 21° C. A spore trap, a length of wide tubing tightly packed with sterilized cotton-wool, is sometimes included in the circuit. It is doubtful whether it assists sterility. Thence the air passes into a glass rake and is distributed into twelve plant chambers, an equal flow through each chamber being obtained by the use of glass capillaries. Owing to the large volume of air moved, it would be inconvenient to use an aspirator, and a gas flow of fairly uniform velocity is obtained by a filter pump. All twelve chambers can be built into a single frame and put into or removed from the thermostat as a unit. The gas stream is led

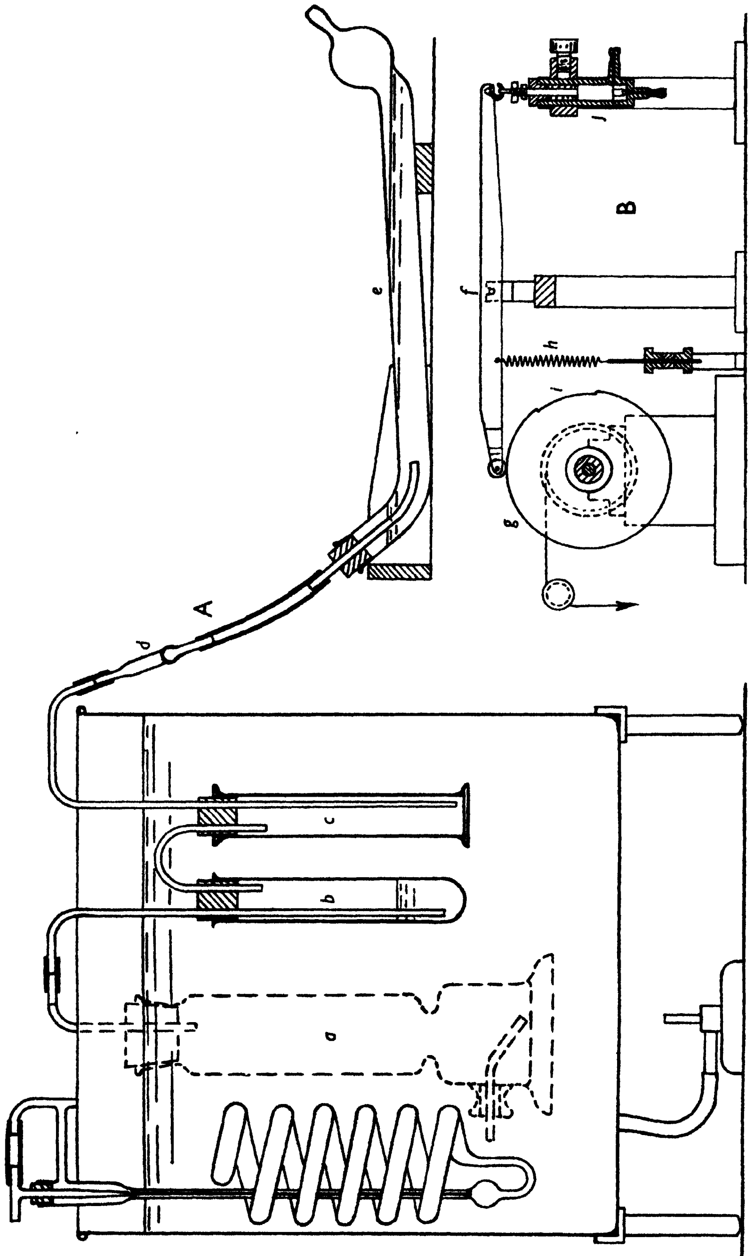
off from near the bottom of the chambers, and normally passes through a corresponding Pettenkofer tube in which the carbon dioxide produced by the leaves is absorbed in $N/5$ $Ba(OH)_2$. A by-pass tube is fitted to each Pettenkofer so that the movement of gas is not interrupted while the Pettenkofer tube is removed for titration of the residual baryta with $N/10$ HCl. In this way temporary accumulations of carbon dioxide in the plant chambers can be avoided.

This apparatus has been used for the simultaneous investigation of twelve separate plant samples of varying cultural histories, etc., and for experiments in which samples are to be removed at successive intervals for examination and analysis. With small numbers of leaves or seedlings results can be conveniently obtained every 12 or 24 hr. over long periods. With larger samples and loss of sleep, 6- or even 3-hourly readings can be obtained for a limited interval.

Apparatus 2

The general nature of this apparatus is the same as that of apparatus 1, but the plant chambers are reduced to two, and an automatic switch is incorporated which diverts the gas stream from one charged Pettenkofer to the next at regular intervals without the presence of the worker. The gas switch (Text-fig. 1 and Pl. 3A) is composed of sixteen cone valves hung in pairs from the long arms of eight levers. Each lever (Text-fig. 1, *f*) rests at its other end on a vertical wheel, *g*, and is pulled down against it by a strong spring, *h*. A slot, *i*, extending exactly one-eighth of the circumference, is cut in each wheel, and when the lever drops into it the longer arm is raised and the two valves attached to it, *j*, opened. The slots on adjacent wheels follow one another in turn, so that one pair of valves is opened at the exact moment when the other is closed. The control wheels are all mounted rigidly on a single spindle (Pl. 3A) which is rotated by a weight attached to a string unwinding from a drum at one end of the shaft. Rotation is slow and discontinuous and is governed by a clock escapement that allows a small forward jerk every half-hour or other desired interval. At every sixth jerk a pair of levers is operated (one up and one down) instantaneously. As usually employed, a change from one pair of Pettenkofers to the next occurs once in 3 hr., and the eight pairs then last for a complete day and night, and titration can be carried out at the investigator's convenience. The air stream is moved by means of a large aspirator tank arranged on the Mariotte principle, so that a very uniform flow is obtainable. In other details this apparatus resembles the first. Its particular use is to allow duplicate samples to be investigated concurrently and to make it easy to secure a continuous record of carbon dioxide output over an indefinite period.

In the earlier experiments using these two apparatuses, the variation (error) due to manipulation covered approximately 0·2 c.c. of 0·1 N HCl. In general it varies round this amount, depending on the care and skill of the worker. According to the rate of respiration, the error to which any individual reading is liable varies from about 1–5 %. Minor irregularities of the carbon-dioxide emission curves could therefore be ascribed to this source, but the main features of the time drift were found to be well outside such limits.



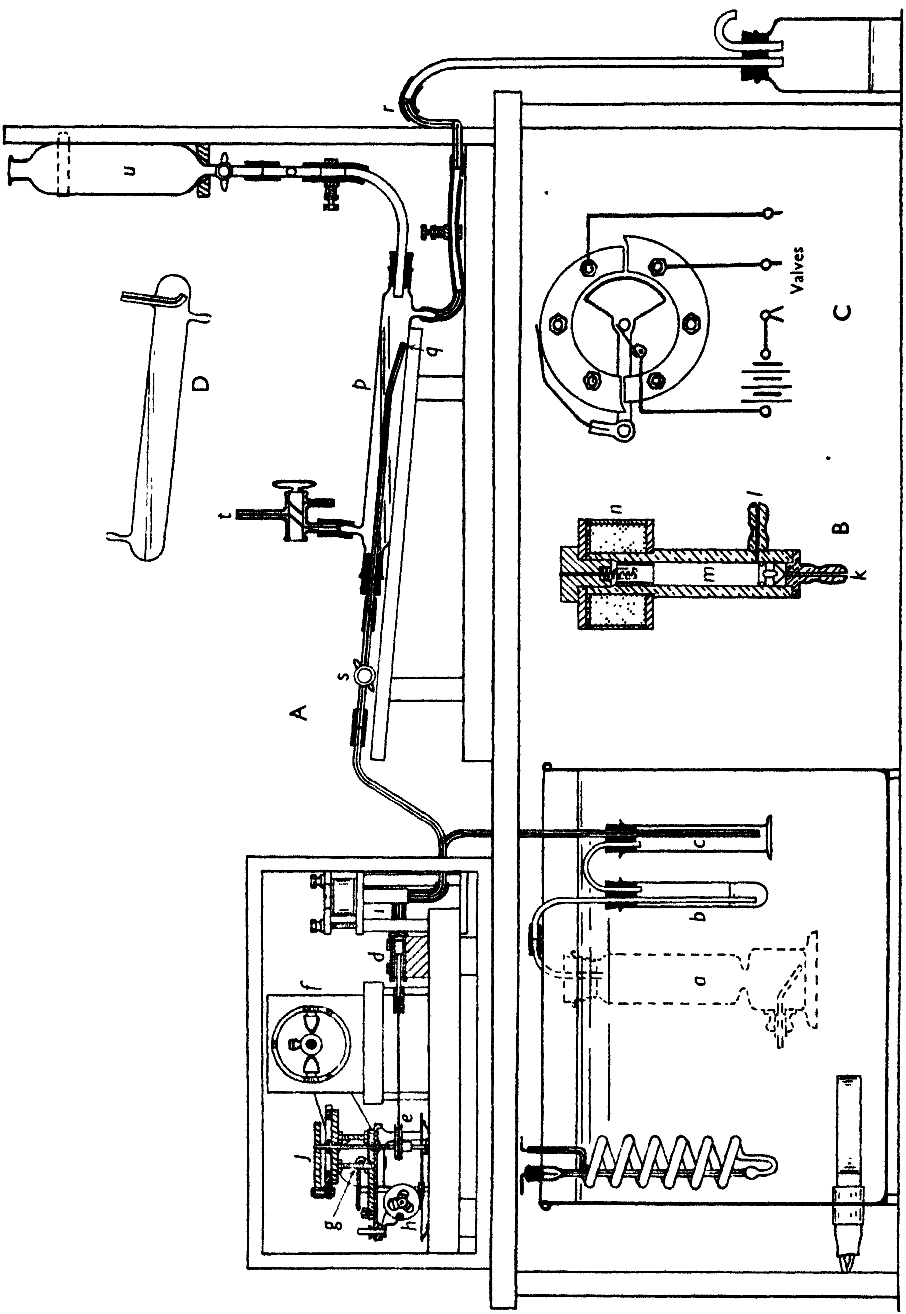
Text-fig. 1. Apparatus 2. *a*, soda lime tower; *b*, baryta tube; *c*, plant chamber; *d*, rake; *e*, Pettenkofer tube. Explanation of other lettering in the text.

Apparatus 3

This consists of the Haldane gas analysis apparatus in its standard laboratory form, combined with an additional device for the semi-automatic collection of gas samples and the maintenance of an air flow through the plant chamber.

The chamber and the vessels leading up to it are of the same pattern as in the other apparatuses, but the air is moved by a pump designed to transfer equal quantities of air during each successive time interval, and deliver it into a prepared receiver. The pump itself (Text-fig. 2, *d*) consists of a 1 c.c. hypodermic syringe with a glass barrel and a bright metal plunger. The thumb rest has been removed from the outer end of the plunger and a connexion made to a rod and eccentric, *e*, giving a suitable length of movement to the plunger. The eccentric is mounted on a spindle which is rotated by a small electric motor, *f*. Great reduction of speed is secured by a worm gear, *g*, and in addition, the speed of the motor, which is liable to a considerable variation if left to itself, is controlled by means of a gramophone friction "governor", *h*. By adjustment of the friction control, the required speed of pumping can be secured, and is accurately maintained over long periods. To direct the gas stream from the plant chamber to the collector, a pair of mercury valves was originally inserted, one on each side of the pump. There was no danger of mercury vapour poisoning the leaves, since the gas stream had left the plant chamber before entering the valves. These valves inevitably had a rather large gas content and required a certain amount of attention from time to time. They were replaced, after a few experiments, by brass cone valves of special design in which the internal air spaces were reduced to a minimum. The present valves are operated by electro-magnets built into the upper end* (Text-fig. 2, *i* and B) and responding to a 4–6 V. circuit made and broken by a commutator attached to the same spindle as the pump's eccentric, *j* and C). Synchronization of pumps and valves is thus assured. In this form the pumping mechanism has been used for months on end with no other attention than occasional oiling. The gas delivered by the pump is collected over mercury in vessels originally having the following construction: The wide tube (Text-fig. 2, *p*) is rigidly mounted, at a slight slope, and the air stream pushed in through the tube, *q*, that opens into the bottom of the receiver. Mercury escapes simultaneously from the overflow, *r*, which is mounted about 1 mm. higher than the opening of *q*. The air is thus pumped in against a constant resistance of a mm. head of mercury. When sufficient gas has been collected, the stream is diverted into a second receiver by turning the three-way tap at *s*. To transfer a sample of the gas to the Haldane apparatus, the overflow, *r*, is closed by its clip, the outlet, *t*, connected to the Haldane, and its tap opened. The air is then driven over by allowing mercury to run into the receiver from the reservoir, *u*. The first sample is rejected through the open arm of the three-way tap at *t*, and a final sample then collected. Alternatively, all leads may be filled with mercury at the start and a sample of air driven to and fro to secure mixing in the collector

* These valves were designed in consultation with S. W. Bush of Oxford, by whom they were made for us.



Text-fig. 2. Apparatus 3. A, Elevation of complete apparatus to scale $\frac{2}{15}$. B, Electrically operated valve, shown in closed position. C, Pump-valve commutator. D, Improved gas receiver, used in later experiments and in apparatus 4. Explanation of small lettering in the text.

before being finally taken into the burette. Analysis is carried out exactly as described by Haldane. When samples are not being collected for analysis, the gas stream can be diverted into a Pettenkofer tube; and it has been shown that estimates of the carbon dioxide output by the two methods give consistent results.

This apparatus has been used to measure the oxygen intake for comparison with the carbon dioxide output, and for calculation of the respiratory quotient (CO_2/O_2). Provided the mercury is kept scrupulously clean, "theoretical" percentages of oxygen are recorded with air that has passed through the empty apparatus; emulsification of the mercury surface with any intrusive impurities has to be guarded against.

Apparatus 4

When sufficient experience of apparatus 3 had been obtained and it was clear that the method gave satisfactory results, the construction of a more elaborate arrangement was undertaken. The principal object was to make the collection of gas samples fully automatic, so that continuous records of carbon dioxide, oxygen, and the respiratory quotient could be obtained simultaneously. It will be clear subsequently that evenly spread readings at suitable time intervals are essential for a proper investigation of the respiratory drifts. The apparatus was also twinned so that duplicate, or control, experiments could be run, as with apparatus 2.

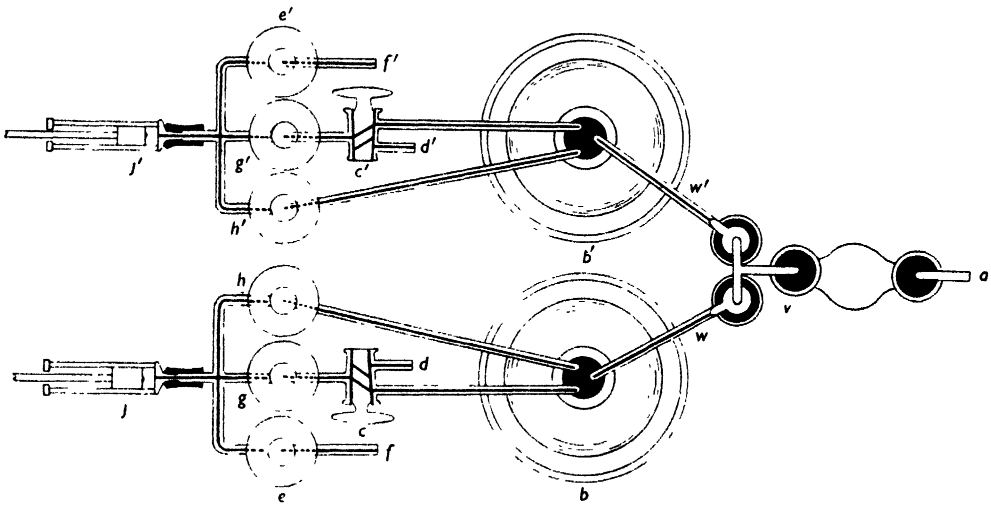
As in apparatus 3, the air used is drawn in from out of doors. A permanent pipe-line has been laid, passing under the floor of the laboratory and up the outside wall to a height of about 12 ft. The air intake is directed downwards, to avoid dust and rain. When the line was first put into commission, a rapid stream of air was kept passing through it for some days until no further absorption of oxygen took place. When gas mixtures other than air are required, a gasometer of the usual floating pattern can be substituted for the outside air by the turn of a tap. The bell of the gasometer is carefully counterpoised so that it does not appreciably compress the gas.

The gas mixture is first led through a scrubbing tower of moist soda lime and a baryta bottle and then through a water valve (Fig. 3, *v*) which is immersed in the constant-temperature bath. This serves to divide the gas stream into two halves, one for each chamber, to keep an even moisture content in the plant chambers, and as a check to back diffusion. Air, once it has entered the chambers, does not come again into contact with this water, being separated from it by a run of about 6 in. of millimetre-bore tubing (Fig. 3, *w*, *w'*). Thus the stirring which is produced in the chambers by the pulsations of the pump does not result in a back-wash of air into the entry tube.

The pumps of this apparatus, one to each chamber, differ from that of apparatus 3 by having a third valve (Fig. 3, *g*, *g'*) in addition to those allowing air to be drawn from the respiratory chamber (*h*, *h'*) and passed into the receivers (*e*, *e'*). This third valve leads to a three-way tap (*c*, *c'*), one arm of which passes back to the respiration chamber and the other to the outside air (*d*, *d'*). If the three-way tap is opened to the chamber a circulation of gas results, and if it is set open to air, some of the gas stream is ejected.

The working of the valves depends on the order in which they receive the 4–6 V. of the valve circuit, and this is determined by the pump commutator and an additional control panel with wander plugs resembling a miniature telephone exchange with three "subscribers".

The succession of contacts (six in number) on the commutator is invariable, but by means of the wander plugs any contact can be connected to any valve. The arrangement commonly used brings the third (return to plant chamber) valve (*g*, *g'*) into operation twice for every single opening of the valve to the receivers (*e*, *e'*). The intake valve to the pump (*h*, *h'*) must necessarily be made to open on every stroke. If the gas circuit is closed at the three-way tap (*c*, *c'*), as it is drawn in Text-fig. 3, this means that each pump-full of gas (approximately 1 c.c.) visits

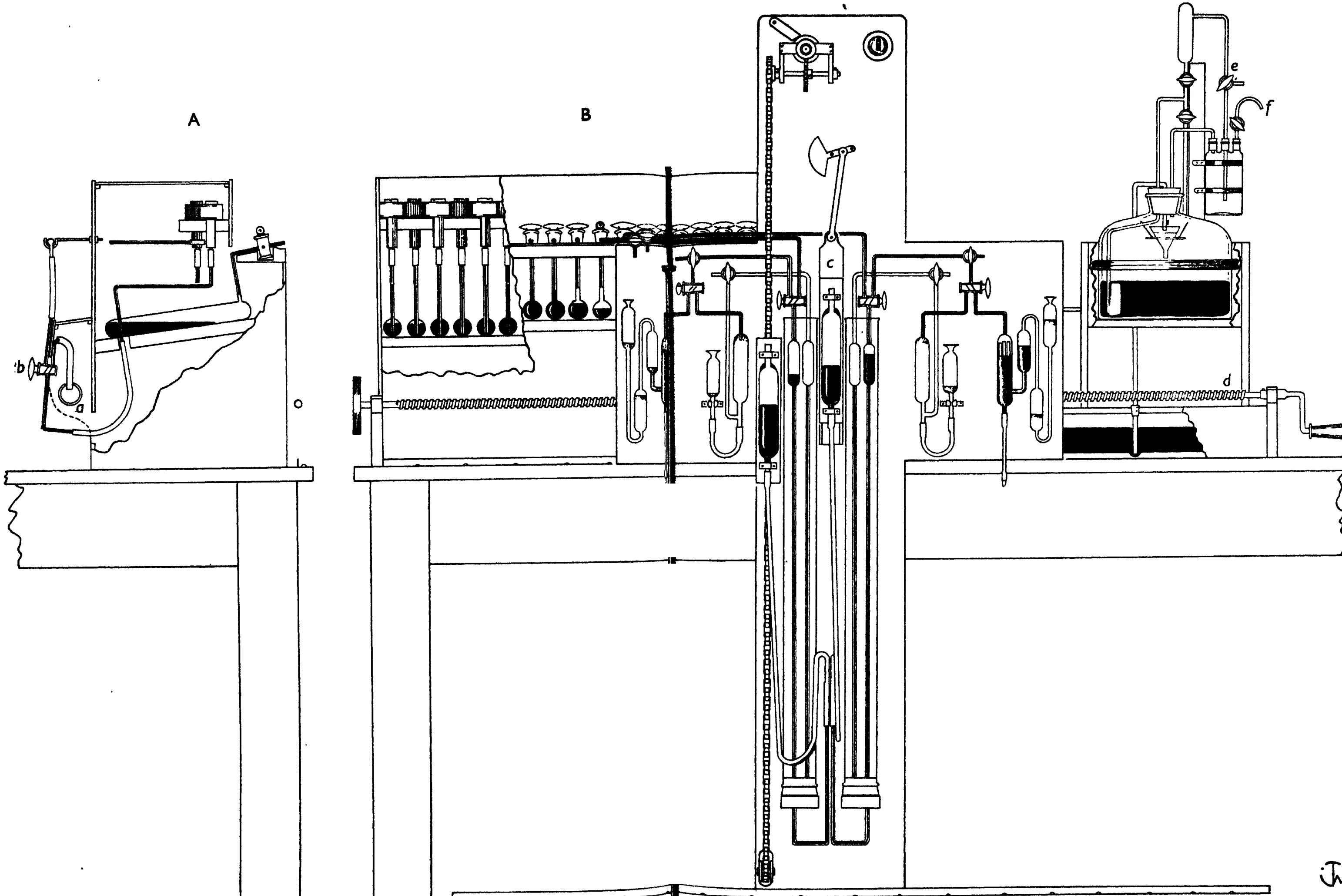


Text-fig. 3. Diagram of the gas leads in apparatus 4. The gas has already passed through a soda lime tower and wash bottle with baryta. The water valve, *v*, consists of a large U-tube (right) with distilled water in the bottom bulb. On the left the gas stream is divided between two vertical chambers whose entry tubes reach to the bottom about 1 in. below a water level. The escape is led off from the top by mm. tubing to the plant chambers, *b*, *b'*. Internal details of the chambers are similar to those already described (James & Norval). Valves *e*, *e'*, *g*, *g'*, *h*, *h'*, as in Text-fig. 2B.

the plant chamber three times in all before being passed on to the receivers. The stirring thus obtained is very valuable in preventing local accumulations of carbon dioxide in the plant chamber.

If the three-way tap (*c*, *c'*) is open, a straight stream of gas is drawn through the plant chamber, two-thirds of which is rejected and one-third passed to the receiver. The amount actually collected is the same with both arrangements; approximately 100 c.c. in each receiver. It is necessary to reject the extra 200 c.c. taken in by the uncirculated stream, as this would exceed their capacity. The circulated gas stream was designed for slow rates of respiration and has proved very useful in the study of the earliest stages of germination. The "straight" stream is employed if a fast respiration rate, which might produce too great a change of gas composition in the circulated stream, is anticipated.

The receivers of the gas samples (Text-fig. 4) are similar to those of apparatus 3,



Text-fig. 4. Apparatus 4. Gas collecting and analysing apparatus × 2/15. A, end view. B, dissected front view. Details in the text.

but their entry tubes are modified, as shown in Text-figs. 4A and 2D, to give greater rigidity. Instead of being controlled by taps worked by hand, they are controlled by electro-magnetic valves of the standard pattern (Text-figs. 4A, 2B). Twelve receiving tubes are provided for each plant chamber (twenty-four in the apparatus), each being connected to its chamber through the arm of a twelve-pronged rake of millimetre-bore tubing attached to the leads (*f*, *f'*) of Text-fig. 3. The control valves are operated by the usual 4–6 V. current passing through the pump commutator and a second, clock commutator. The latter has a face of twelve contacts, over which the travelling contact is moved by the clock once in 24 hr. As a result of this arrangement each receiver valve synchronizes with the output valve of the pump for 2 hr., after which it remains closed and the next takes up the tale. At the end of the 24 hr. a set of twelve samples has been collected for analysis (twenty-four if both chambers are in operation).

Text-fig. 4A (end view) shows a receiver collecting a gas sample and displacing mercury through the right-hand prong of the tap *b*, whose level is adjusted just below the intake of the receiver. The displaced mercury drops into the wide tube *a* and flows down to the sump, a heavily built oak box shown on the extreme right of Text-fig. 4B. From this the mercury is raised by exhaustion into the thick glass reservoir immediately above as occasion requires. Each time the mercury is raised it passes through perforated filter-paper in the suspended funnel. The reservoir provides enough head for the mercury to flow back into the gas receivers when the tap *b* is turned over, but not enough to force it into the valves which are mounted on the bridge shown immediately above the receivers. Connexion is through a siphon (Text-fig. 4B, top right), which can readily be made by turning the tap *e*, whose side limb leads to a filter pump, and broken by admitting air through *e* and *f*. This relieves the mercury pressure on all taps and joints, except when it is actually required, and is a necessary precaution against losses of mercury.

The gas analyses of twenty-four samples is a lengthy undertaking, and the following modifications have been introduced to economize time. Two Haldane apparatuses are mounted side by side on a vertical carriage, one of them being specially constructed "left-handed" (see Text-fig. 4B). On the middle of the panel between them a teak and brass carrier *c* works in brass slides. It is moved up and down by a small electric motor, fixed to the back of the carriage, with suitable gears and transmission. The mercury reservoir of either Haldane (the right-hand one in Text-fig. 4) can be hooked on to this carrier, and when the motor is started the gas sample is moved to and fro in the absorption pipette. Most of the time taken by a Haldane analysis goes in completing the oxygen absorption in the pyrogallol, and other absorbents do not give better results.* With the mechanical stirring described above, while absorption is going on in one apparatus, a sample can be drawn into the other by lowering its mercury reservoir on the chain (Text-fig. 4B, left-side

* Dr J. S. Haldane told us in conversation that, in spite of further work, he had not been able to find a better oxygen absorbent than pyrogallol, well matured by long keeping in the absence of air and light.

Haldane) and the carbon dioxide absorption carried out. The saving of time on each pair of analyses is 30 %.

The vertical carriage supporting the Haldane is hung on rails and controlled by a long screw (Text-fig. 4, *d*). It can thus be racked into position opposite each receiver and connexion made to the receiver taps with the minimum loss of time. When the sample is complete, the tubes are disconnected and a couple of turns of the screw brings the carriage opposite the next receiver. In Text-fig. 4B the tenth receiver is being sampled into the left-side Haldane and the ninth awaits final refilling with mercury. The third to the eighth have been reset, and gas collection is going on in the first two.

With these helps a practised manipulator can do the twenty-four analyses in less than 6 hr.; and a considerable number of respiration records, continuous over several days, have now been accumulated.

Certain results obtained with these apparatuses have already been published (James & Arney, 1939; James & Norval, 1938, apparatuses 2 and 4; James & Hora, 1940, apparatus 4; Yemm, 1935, apparatuses 1, 2 and 3).

We are indebted and wish to express our gratitude to the Christopher Welch Trustees for a series of substantial grants, which has covered the building of most of this apparatus, and also a maintenance grant to A. L. James. The building of the apparatus was greatly helped by the technical skill of Mr A. Mitchell.

The plant chamber

The form of the plant chamber has been varied to suit the circumstances of the experiment. In the present series, a conical pyrex flask, with inlet and outlet tubes, and a layer of prepared sand at the bottom was used for the first experiments (see Pl. 3B). Later, the domed chamber already described by James & Norval (1938) was substituted on account of its convenience.

RESULTS

Carbon-dioxide emission by seedlings from entire grains

This was first examined by the use of apparatus 2. Two simultaneous experiments were run, one of which was started 24 hr. after the other, by utilizing the two gas circuits of the apparatus. The plant chambers were conical pyrex flasks of about 250 c.c. capacity, painted black on the outside. A layer of silver sand, purified by treatment with hydrochloric acid and frequent washings with distilled water, was placed at the bottom of each flask. The flask with its contained sand was sterilized by heating to 120° C. for 30 min. on each of three successive days. The rubber stopper with its outlet and inlet tubes were sterilized with formalin washed away with sterile water. The sand was moistened with a complete culture solution (made up as in James & Norval, 1938) which had been kept at 100° C. for 30 min.

Eighty barley grains of the 1933 harvest were surface sterilized with absolute alcohol for 2 min. and then washed thoroughly with sterile water, and immediately

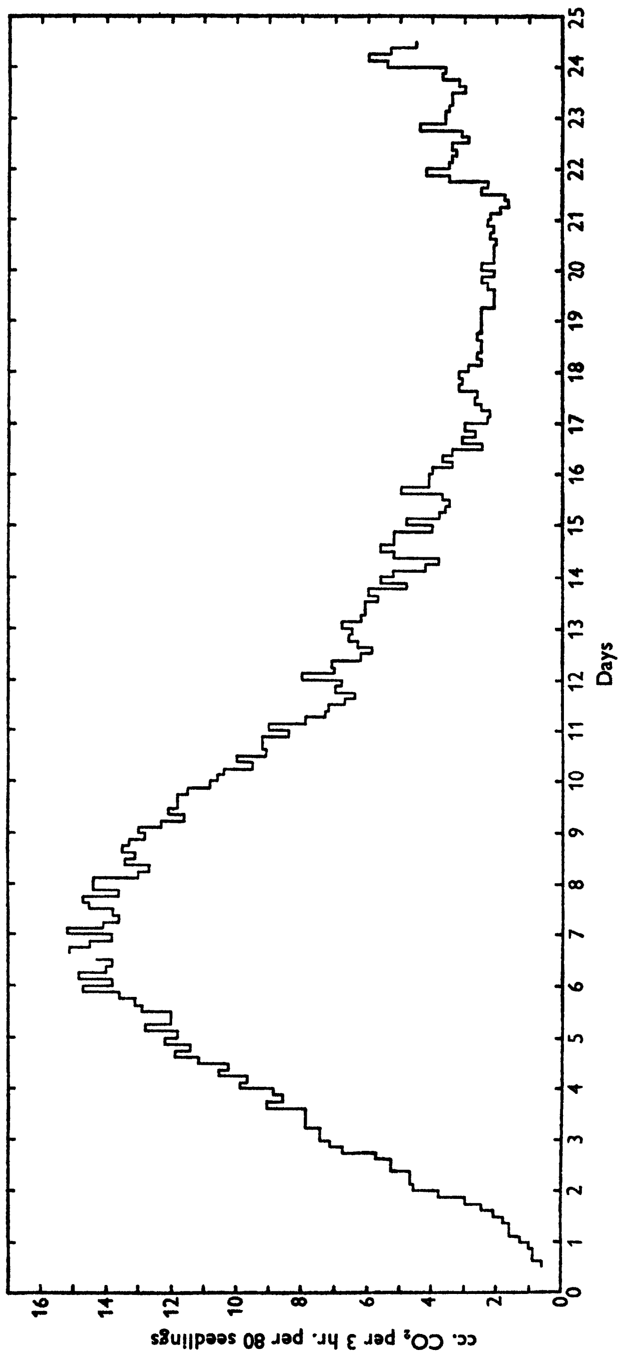
introduced into the plant chamber and pressed down on the surface of the sand. The set-up used in this experiment is shown in Pl. 3B.

The carbon dioxide emissions for each 3 hr. period are recorded in Text-fig. 5 up to 588 hr. after the grains came into contact with the culture solution. During this period the plantlets have passed from the dormancy of the grain, through an embryonic stage and a much shortened "maturity" into senescence and death. The corresponding drift of carbon dioxide output falls into five phases, clearly recognizable in Text-fig. 5. *Phase* 1 shows a rapid increase in the rate, and lasts for 2 days, when it passes somewhat abruptly into *Phase* 2 showing a noticeably slower acceleration. *Phase* 2 concludes with the achievement of a maximum some time on the seventh day, and passes almost at once into *Phase* 3. Here the rate of carbon dioxide emission falls off with increasing rapidity until the twelfth day. An interruption then takes place, and in *Phase* 4 the rate falls off slowly and rather irregularly to a minimum. During the twenty-second day *Phase* 5 begins with a small rise. These divisions of the experimental sequence are represented schematically in Text-fig. 16, p. 175.

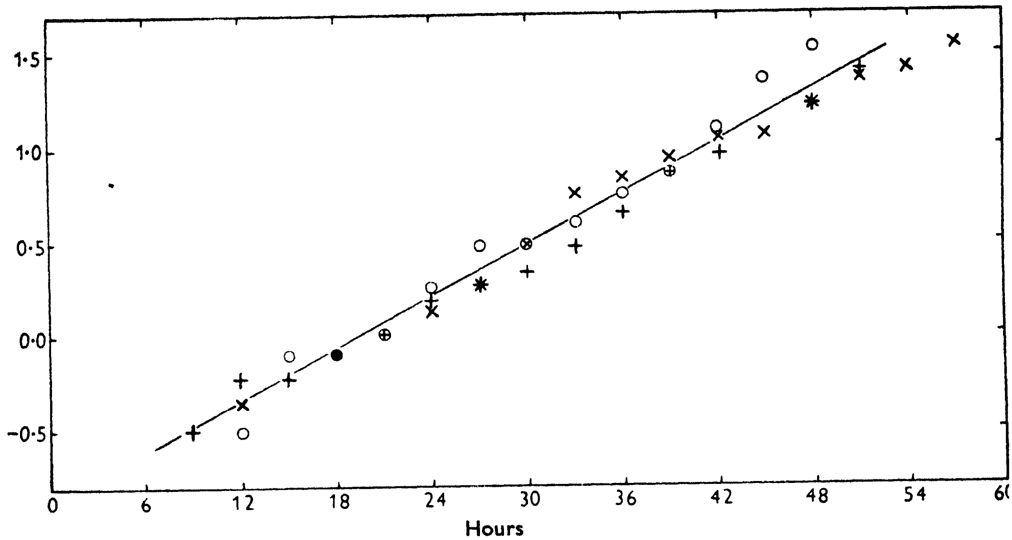
Phase 1. Experiments covering the first three phases have often been repeated not only by ourselves but also by other workers in the Department, and there is no difficulty in recognizing them. Further experiments with grain of the 1933 harvest showed an almost identical drift in phase 1, which is a very close approximation to an exponential increment (see Text-fig. 6). The straight line obtained by plotting the logarithms of the rates may be extrapolated back to $t=0$, i.e. the moment of bringing the grain into contact with water. At first sight this would appear to represent the log rate of the dormant grain. The rate of CO_2 emission thus obtained is 0·43 c.c., CO_2 per 3 hr. per 80 grains (Text-fig. 7). Taking "dry matter" $=0{\cdot}64$ CO_2 (by weight) this would represent a loss to each grain of 0·21 mg. dry weight per annum, only slightly less than half the total. Even allowing for an experimental temperature a few degrees higher than the mean storage temperature, it is evident that the actual dormant respiration must be considerably lower than the estimated. Experiments carried out subsequently (1934 harvest, apparatus 4) included readings during the first 6 hr., and revealed a very marked depression of the initial values (Text-fig. 7) below those of the extrapolated curve. The depression is not due to apparatal lag, since it has been found that if this exists at all it is not detectable after 4 hr.

The 1934 figures differed from the 1933 in maintaining a more rapid acceleration of the CO_2 output. They changed over from the first to the second phase at the same rate, however, i.e. 4·0 c.c. CO_2 per 3 hr. for the 80 grains. Owing to the more rapid acceleration they reached this rate after about 30 hr., as compared with the 48 hr. of the 1933 grain. Plotting the rates as logarithms throws both the ending of phase 1 and the opening of phase 2 into linear form, and the transition is then revealed as a sharp break in the curve (Text-fig. 7) for each set of grain.

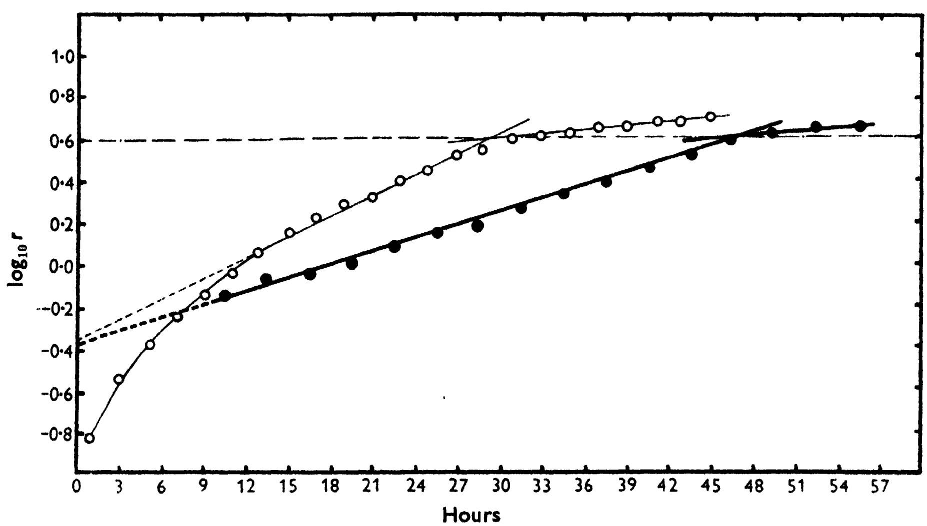
Phase 2. The principal variations observed are in the actual magnitude of the maximum and the time taken to reach it. The former has varied from about 14 to 17 c.c. CO_2 per hr. per 80 seedlings; and the time taken to reach the maximum from



Text-fig. 5. The carbon dioxide output of eighty seeds and seedlings of 1933 harvest, germinating on sand moistened with a complete mineral culture solution. Means of two experiments, one started 24 hr. after the other.



Text-fig. 6. $\mathrm{Log}_{10}\, r$ (= common log of the CO_2 emission rate of seedlings) plotted against time during the first 50–60 hr. of germination. The three different symbols represent three successive experiments, all with 1933 grain.



Text-fig. 7. $\mathrm{Log}_{10}\, r$. Plotted against time during the opening stages of germination. ●——● 1933 grain, mean of three experiments shown in Text-fig. 6. ○——○ 1934 grain, mean of four experiments. The point of inflexion of the curves gives the point of transition from phase 1 to phase 2, and is determined by the respiration rate, not by the duration of germination alone.

about 6 to 8 days. The most usual time has been about 6 days (cf. James & Arney, 1939, Fig. 1), though the 1933 grain (cf. Text-fig. 5) took 7 days. The approach to the maximum rate is always gradual and passes smoothly into the decline of phase 3. We have never observed a relatively abrupt transition to a uniform rate which is then maintained for considerable periods, as recorded by Barnell (1937) for Spratt Archer.

Phase 3. In Text-fig. 5 this is symmetrical with phase 2, the two forming a parabola fitting the equation $R-r=0{\cdot}5\ (T-t)^2$, where R is the maximum respiration rate at time T, and r the rate at any time t. In subsequent experiments symmetry about the maximal rate was not observed, the deceleration of phase 3 being less than the acceleration of phase 2. It is to be noted, however, that the deceleration becomes increasingly rapid until interrupted by the check of phase 4.

Carbon-dioxide emission by seedlings from excised embryos

In 1890 Brown & Morris showed that it was possible to germinate embryos isolated from their natural source of nourishment by removal from the seed. They brought the seedlings to maturity by supplying them artificially with nutrient salts and carbohydrates during the early stages. They also found that seedlings given no carbohydrates will grow for about 10 days.

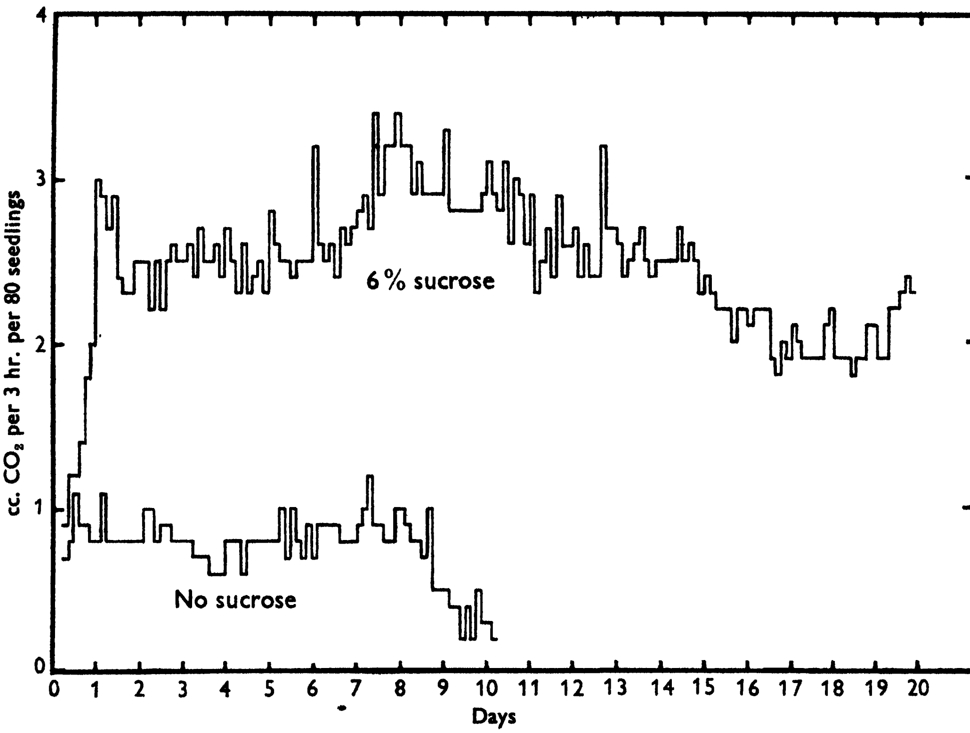
In order to follow the carbon-dioxide emission of such seedlings, eighty embryos were excised and germinated, as in the previous experiments, in a dark chamber on sand moistened with a normal culture solution, without sugar. The embryos are not in any way damaged during the extraction, nor is it necessary to cut them away from the endosperm nor to interfere with the scutellum. The grain is first soaked in water for about 3 hr. The inferior palea is then removed, when the embryo becomes clearly visible through the testa and pericarp. These are cut with a sharp flamed scalpel and the embryo can then easily be removed. There is no organic connexion to sever between the scutellum and the endosperm. It is pointed out by Brown & Morris that during the formation of the embryo, when it is increasing in size, starch is withdrawn from adjacent cells, emptying them of their contents. These cells die and their walls are crushed between the growing embryo and the remaining starch-containing tissues of the endosperm, forming a non-living layer not organically continuous with the scutellum. It is thus possible to remove the embryo cleanly and without injury.

Great care is necessary to insure sterility from moulds, yeasts and bacteria. The use of antiseptics, whether liquid or volatile, during the act of dissection is ruled out, because both respiration and germination are already affected by concentrations too low to destroy contaminants.

The use of a sterile room and box was not found to be of much assistance, the main source of infection being the surface of the grain, not the atmosphere surrounding it at the moment of operation. Ordinary precautions of cleanliness of bench and hands were found equally satisfactory and the grain sterilized as before; during the period of soaking it was completely immersed in sterile water. The embryos were detached by the point of a flamed scalpel and immediately transferred

to the prepared plant chamber. They were not touched by hand. In the experiments now to be reported no signs of infection were visible at the end, nor does the form of the carbon-dioxide emission curves suggest a multiplication of micro-organisms. The use of moistened sand as a medium is of great assistance in maintaining sterility during such experiments: it has been found to be much less favourable to micro-organisms than a freely exposed liquid surface.

The experiment was carried out with apparatus 2, exactly as in the previous experiments with whole grains, except that a smaller plant chamber of about 100 c.c. capacity was used. The time curve of carbon-dioxide emission is shown in Text-fig. 8.



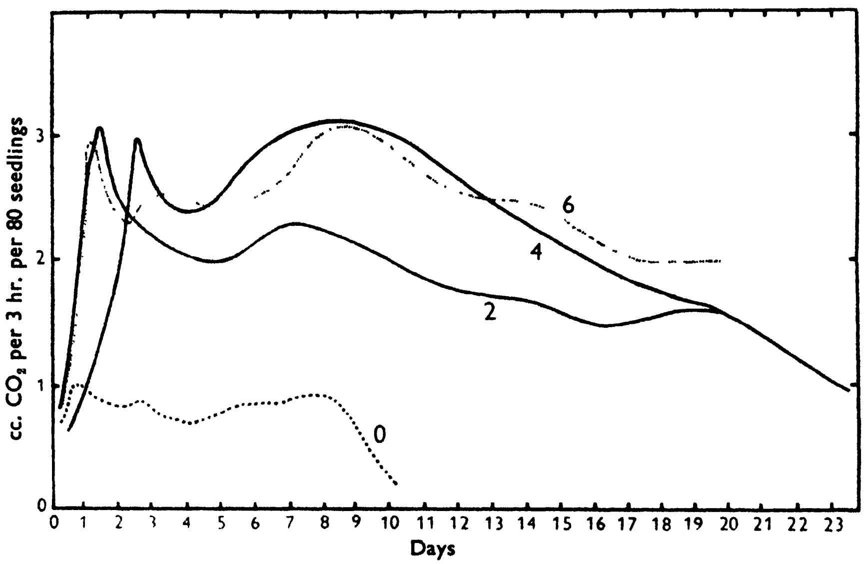
Text-fig. 8. The CO_2 output of 80 embryos separated from their endosperms and germinated on sand moistened with a complete mineral culture solution. The lower curve refers to an experiment without added sugar; the upper curve to an experiment including 6 % sucrose.

Brown & Morris found sucrose the best sugar as a substitute for the endosperm, with an optimal concentration of about 4 %. The foregoing experiment was therefore repeated three times, with concentrations of 2, 4 and 6 % sucrose added to the culture medium. The results are also shown in Text-figs. 8 and 9, and a set of embryos photographed on the fifth day is shown in Pl. 3C.

The embryos without added sugar only increase their rate of carbon-dioxide output during the first day, and for the next 4 days there follows a decline. There is then a slight secondary rise until the eighth day when a fall sets in, leading to

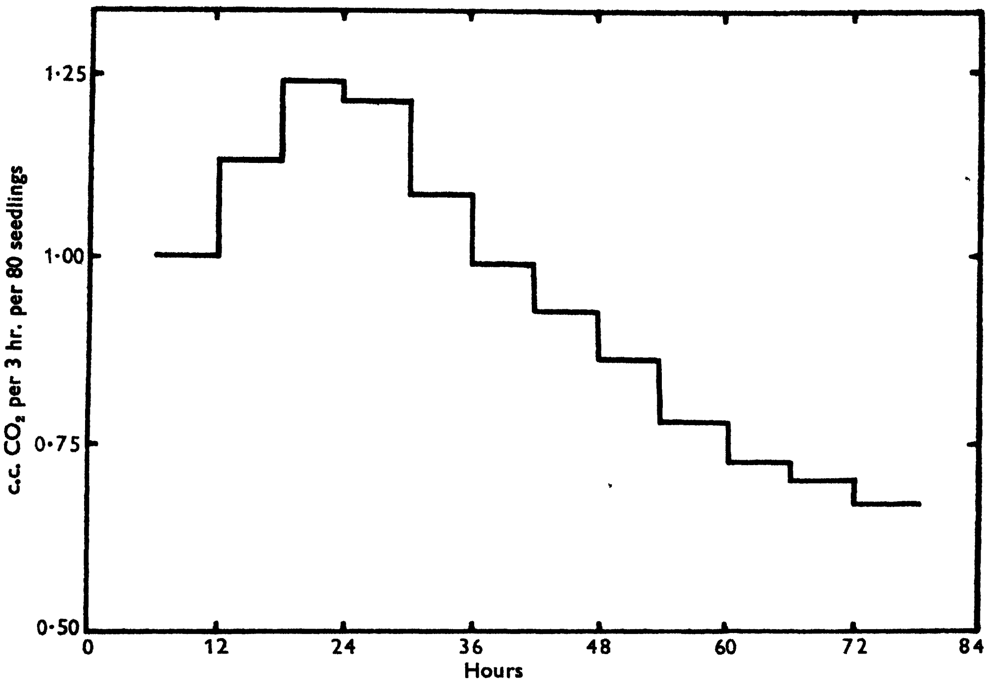
death. The mean rate is so low that any more definite drifts are obscured by the experimental error.

An experiment using 120 embryos—instead of 80—and 6-hourly collections of the carbon dioxide, showed the nature of the initial stages more clearly (Text-fig. 10). The corresponding stages in the presence of sucrose are similar, but continue to a higher level. After the first rapid rise there is a temporary decline. With 2 % sucrose the second maximum is still not very pronounced, and the curve passes into a long-drawn phase of slowly declining carbon-dioxide output at about twice the rate obtained in its absence, and much longer sustained. There is a better defined second maximum with both 4 and 6 % sucrose round the ninth day.

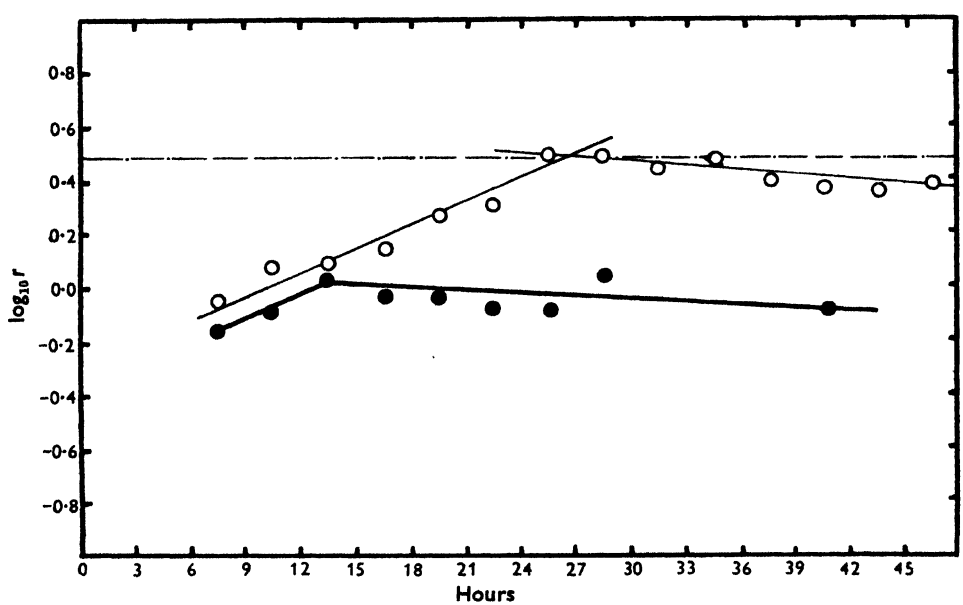


Text-fig. 9. The drift of CO_2 output of excised embryos receiving complete culture solution and percentage additions of sucrose, as shown by the figures against the curves. Individual readings are omitted for the sake of clearness, but are shown for the 0 and the 6% curves in Text-fig. 8.

Comparing these drifts with that of the complete grain reveals certain similarities. In each there is a period of rapid acceleration (phase 1). Within their limitations, the data suggest the existence of an exponential phase of increment, which reaches its limit at 3·0 c.c. CO_2 per 3 hr. per 80 embryos fed with sucrose (Text-fig. 11). If the phase exists at all in the unfed embryos, it comes to an end at a much lower value. The transition from phase 1 to phase 2 reveals itself in the entire grains as a rather sudden reduction of acceleration; but in the isolated embryos there is an actual drop in the rate itself. This is a feature of great interest for the interpretation of these drifts (see p. 172). Phase 2 shows itself in the embryos fed with 4 and 6 % sucrose as a slow rise, as in the entire grains. It only attains about a fifth of their maximal rate, and the 6 % sucrose shows no advance on the 4 %. The slow decline that follows cannot be subdivided, and phases 3 and 4 merge. It will further be shown from the evidence of the R.Q. and carbohydrate analyses



Text-fig. 10. The CO_2 output of 120 embryos on mineral culture solution without sucrose. The rates are reduced to c.c. per 80 embryos per 3 hr., for comparison with Text-figs. 8, 9.

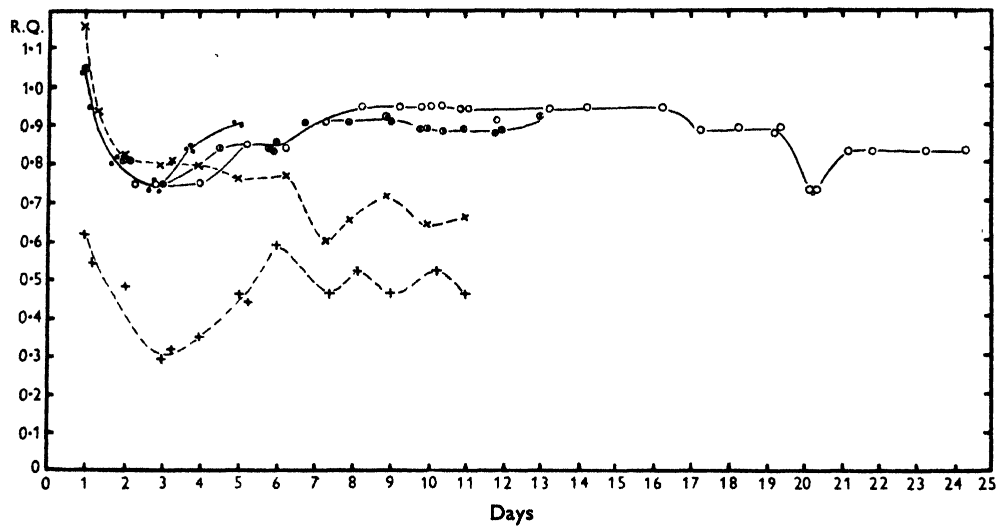


Text-fig. 11. $\text{Log}_{10}\, r$ of embryos plotted against time from data given in Text-fig. 8, ●——● without sucrose, ○——○ with 6% sucrose.

that the characteristic of phase 4, i.e. protein degradation, invades even the second phase which is no longer supported almost wholly by sugar consumption, as in the entire seed.

The respiratory quotient of the dormant grain

In the study of the respiratory quotients, that of the dormant grain was found to be of great importance as a point of departure. It was determined as follows. A 3 l. jar with a rubber stopper and entry tube closed by a clip was exhausted and brought back to atmospheric pressure in the open air. It was about half-filled with grain, still in the open, and then stoppered. It was allowed to stand in the laboratory at room temperature from 26 June to 28 July, and was well shaken every few days to prevent local accumulation of carbon dioxide among the grains. At the end of



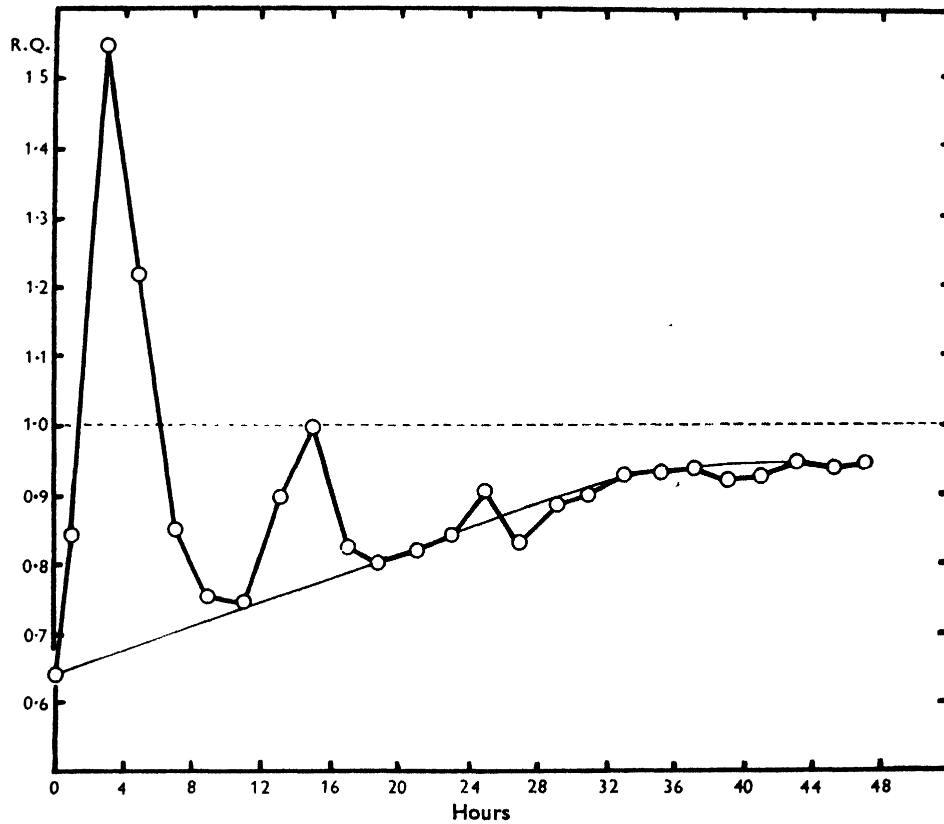
Text-fig. 12. Drift of the R.Q. of seedlings of 1933 grain. Experiments with entire grain are given with continuous lines; three separate experiments shown by ●, ○, ⊙. Experiments with excised embryos are given by broken lines. Embryos without sucrose, + ; with sucrose, ×. The experiment marked with hollow circles, ○, was continued until the seedlings decomposed, the R.Q. showing a slow fall to 0·5.

the period, a sample of gas was withdrawn and analysed in the Haldane apparatus. The experiment was carried out three times in all, and the values of the R.Q. recorded were 0·67, 0·60, 0·66; mean = 0·64.

The respiratory quotient of seedlings from entire grains

This was first examined with samples of the 1933 grain, using apparatus 3. Eighty grains were planted in a Petri dish with prepared sand and culture solution as before, and enclosed in the usual domed respiration chamber. Sterilization was carried out exactly as in the previous experiments. For the greater part of the time the gas stream was pumped to waste, but samples were collected and analysed as it was convenient. Three successive experiments were carried out, giving the curves of Text-fig. 12. These results are not unlike the classic results of Bonnier & Mangin

(1884) with wheat. There is, however, one difference which, on account of the interpretation that has been put upon Bonnier & Mangin's data, is important. Our earliest values are greater than 1, and the form of the curve suggests that still higher values may have obtained at an earlier stage. With the completion of apparatus 4 it was possible to investigate these early stages more thoroughly, using the grain of 1934. Values up to 1·55 were then recorded. The complete time drift of the R.Q. (Text-figs. 12, 13) is complicated. It begins with a steep rise from the



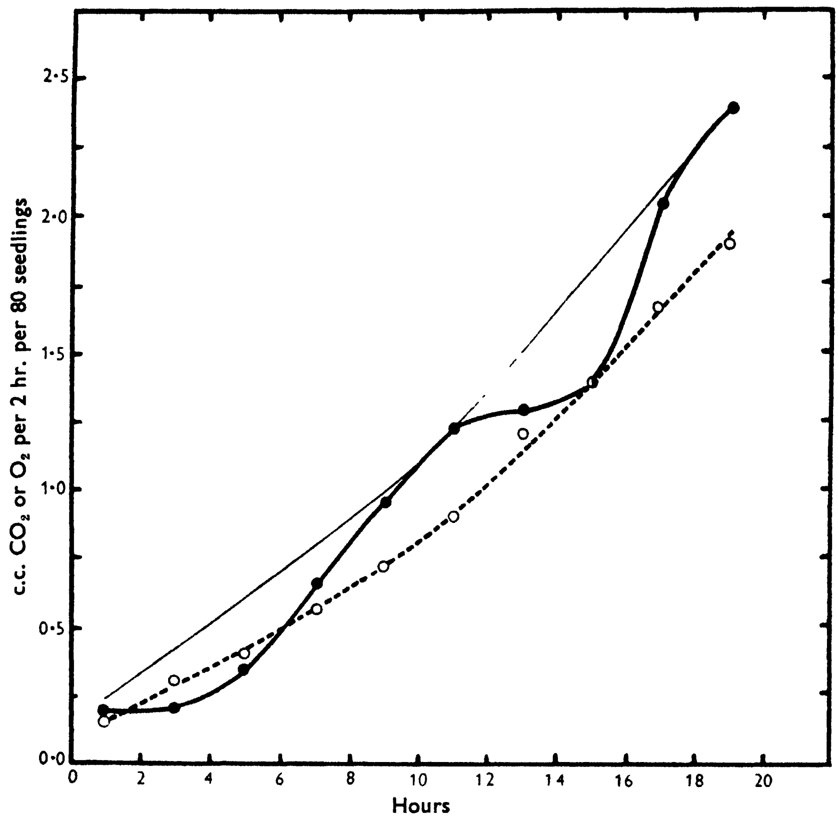
Text-fig. 13. Drift of the R.Q. of seedlings of 1934 grain. Mean of four experiments, except the initial value—that of the dormant grain—which is a mean of three experiments. The thin line is drawn as a simple transition between the "dormant" and "steady" values (cf. Text-fig. 14).

low value of the dormant grain, passing rapidly through a maximum back to a value little above the original. There are then further minor oscillations superimposed on a slow rise to a value of 0·90–0·95. This is maintained for a prolonged period and passes eventually into a slow decline (Text-fig. 12).

Attention may be called to the different time scales of the 1933 and 1934 grain. At the end of 50 hr. the 1933 grain was only approaching its trough value; but the 1934 grain had passed through these initial stages and established its steady value. The oscillations of the R.Q. are not determined by the carbon dioxide drift and have

no constant relation to its successive stages. Thus the R.Q. 1934 had almost achieved stability by the end of phase 1, but the R.Q. 1933 was rising to a corresponding condition throughout phase 2.

In Text-fig. 14 the carbon dioxide emission and oxygen intake of the 1934 grain are plotted simultaneously. It is clearly shown that the temporary rise of the R.Q. is the result of an oxygen lag rather than of the sudden release of accumulated carbon dioxide.



Text-fig. 14. CO_2 output ○---○, and O_2 intake ●——●, from the same experiments as Text-fig. 13. The thin continuous line shows what the oxygen uptake would be if the R.Q. followed the simple course shown as a thin line in Text-fig. 13.

There are thus four main features of these drifts to be considered: the general rise from the low R.Q. of dormancy; the fluctuations superimposed on this rise; the steady R.Q. (0·9–0·95); and the final decline. The following sections provide further data for the interpretation of these phases.

The respiratory quotient of excised embryos

Experiments were performed with 1933 grain, using apparatus 3. The embryos were dissected out as before and planted on prepared sand with the complete culture solution, but no sugar. Determinations of the R.Q. were made until the

grains succumbed after 11 days. The curve (Text-fig. 12) follows a similar course to that of the complete grain, but is pitched lower. It falls throughout the second and third days, and rises from the third to the sixth. It then becomes comparatively steady round 0·5. The relation between the two curves shows that the causes of the R.Q. fluctuations are resident in the embryos, not in the endosperms, and are unaffected by the act of removal. A second point of interest is the lowness of the minimum: R.Q. = 0·29. In an experiment with 1934 grain already published (James & Norval, 1938), the R.Q. of excised embryos sank even lower. Values between 0·25 and 0·23 were maintained for a couple of days.

A similar experiment, with 6 % sucrose added to the culture medium, was made with the 1933 grain and continued for 11 days also (Text-fig. 12). The presence of the sugar was at first able to raise the R.Q. to that of the entire grain. This was due to an increased output of carbon dioxide, not to a reduction of oxygen absorption. There was, however, no rise from the trough value, and the R.Q. sank slowly to values between 0·7 and 0·65.

Comparison of these various curves shows clearly that the trough values for the entire grain are composite. It has been shown directly (James, 1940) that the breakdown of carbohydrate (R.Q. = 1, if complete) is taking place in the embryo from the very beginning. The R.Q. values of the isolated embryos with their minimal carbohydrate content are too low to be due to oxidation of fat or protein, and show that incomplete oxidations must be occurring simultaneously. By the addition of sugars, artificially from outside or naturally from the endosperm, the R.Q. is raised. The incomplete oxidations are not suppressed, but continue simultaneously with utilization of the sugar, since a compromise R.Q. lying between 0·23 and 1·0 is obtained.

The ether-soluble fraction

It seems improbable that the depression of the R.Q. during dormancy and the early stages of germination is due to accumulation of incompletely oxidized products of carbohydrate breakdown, such as the organic acids. The addition of further sugar would not then be expected to raise the R.Q. as it does, but would merely lead to increased acid production. In addition, the consumption of such substances as starvation advanced would cause the R.Q. to rise above 1·0, and no such rise occurs.

The most probable reaction would seem to be a conversion of fats involving an intake of oxygen without any corresponding evolution of carbon dioxide. This could not be open to the same objections as acid formation; and might proceed in parallel with carbohydrate respiration as suggested by the R.Q. experiments. Barley grains usually contain about 2 % of fat, and an attempt was made to see whether the quantity did in fact become less during dormancy and germination.

The accurate determination of the ether-soluble matter presented considerable difficulty. After a number of trials, the following "Dry Soxhlet" method was adopted. 100 barley grains, after removal of the paleae, were weighed and then crushed in a hydraulic press at 2 tons per sq. in. They were then transferred to a glass evaporating dish and covered with boiling alcohol. The dish was put into a vacuum desiccator which was exhausted, and the air pressure returned to drive the

alcohol into the tissues. The effect of the alcohol is to render soluble lipoids which are otherwise only partly extracted by ether. The alcohol and moisture in the grain were removed by 21 hr. drying *in vacuo* over calcium chloride at 60° C. The dry material was ground in a small mortar and transferred to a Soxhlet thimble, previously extracted with ether. The thimble was put into the body of a Soxhlet extractor of standard pattern. The evaporating dish in which the grain had been dried was washed with three rinsings of ether which were poured on to the ground material in the thimble. The residue adhering to the dish was white and readily soluble in water and could therefore be discarded.

The Soxhlet extractor was then assembled. All joints were of ground glass and were not greased. The air vent of the reflux condenser was fitted with a calcium chloride tube, to prevent the access of atmospheric moisture. The lower flask of the extractor containing the ether was placed in an electrically controlled water-bath, and extraction continued for 5 hr. The material was then taken out, reground in the mortar and returned, the last traces being swept back into the thimble with a fine brush. Extraction was repeated four times in all, each time for 5 hr. The extract stood in the Soxhlet overnight as necessary, but the heat was turned off. A fifth extract contained only an unweighable trace of fats.

When the extraction was complete, the ether was evaporated off from the flask and the extract taken up with petrol ether. A little fine starch was occasionally present in the extract, and was removed by passing the petrol-ether solution through a hardened filter paper (Whatman 50). It was collected in a weighing bottle; the petrol ether evaporated off, and the residue dried for 30 min. in an oven at 80° C. It was then cooled in a vacuum desiccator and weighed.

Estimations were first made on samples of grains (1933) taken direct from the bin, exactly as used for the respiration experiments. Estimations were also carried out on samples that had been spread out and exposed at room temperature for a further 15 weeks (28 August–11 December 1934); and, finally, on grains that had been germinated in apparatus 4 for 50 hr. The last were treated exactly as in other respiration experiments, except that the sand was extracted with ether before use, as an additional precaution.

Table 1. *Milligrams of ether-soluble material in* 100 *grains*

Grain direct from bin, August	Seedlings after 50 hr.	Grain exposed dry until December
114·5	116·2	112·3
118·5	112·9	108·4
115·5	113·5	
	111·1	
Mean 116·2	Mean 113·4	Mean 110·4

The samples of grain were graded to weigh 5·00 g., so the content of ether-soluble material only slightly exceeds 2 % in each category. Both germination and the slow processes of dormancy appear to reduce it, though only slightly. Such slight reductions would, however, be enough to account for the additional uptake

of oxygen observed, even assuming only a moderate degree of oxidation. The limitations of the extraction method and the variability in the amounts of excess oxygen absorbed in successive germinations prevent a quantitative comparison between the loss of extract and the excess of oxygen absorbed over carbon dioxide emitted. A loss of ether-soluble material seems to be established, but we have not yet succeeded in establishing its amount.

The formation of cutin

The ether extract is fluid at room temperature and browns on prolonged exposure to the air. Nevertheless, oxygen storage in the extract itself does not seem to be of importance during germination, since its percentage of carbon slightly increases instead of diminishing. These percentages, determined by a Pregl micro-combustion, were 62·2 before germination and 75·0 after.*

The oxidation of certain fatty acids is an important process in the formation of the complex mixture, cutin (Lee, 1925). Cutin is insoluble in ether and such an oxidation would, therefore, lead to a loss of ether extract and, at the same time, oxygen would be absorbed without any corresponding production of carbon dioxide. The formation of cutin was found to occur in germinating embryos over the surface both of the coleoptile and of the first leaf rolled within it. Contrast was afforded by the roots, on which no such formation could be observed. The leaf and coleoptile behaved so much alike that separate description is unnecessary. Both have unwettable surfaces, in contrast with the readily wetting surfaces of the roots. On dissolving the leaf and coleoptile in strong sulphuric acid an insoluble brown residue was formed. On treatment with chlor-zinc-iodine, the surface gave a yellow-brown reaction at once, and the blue cellulose reaction developed more slowly, and first of all where the interior was opened by tearing of the tissues. When the blue colour was fully developed, the yellow cutin reaction was still sharply visible at the leaf edge. The roots gave an immediate blue colour without any development of surface yellows. After boiling with 15 % KOH, the resistant surface was removed; the cells tended to fall apart and gave a blue surface reaction directly, the cuticle having been dissolved. The tissue surface also took up colour from a strong alcoholic solution of chlorophyll, and from a solution of Sudan IV. Material was also put into Sudan IV after being extracted overnight with ether. Little was then absorbed by the cell contents; but the surface walls took up the dye strongly. Edge walls in optical section clearly showed both stained cutin and unstained cellulose layers; the two layers were of about equal thickness. It thus seems evident that considerable quantities of cutin are formed progressively as the seedling enlarges.

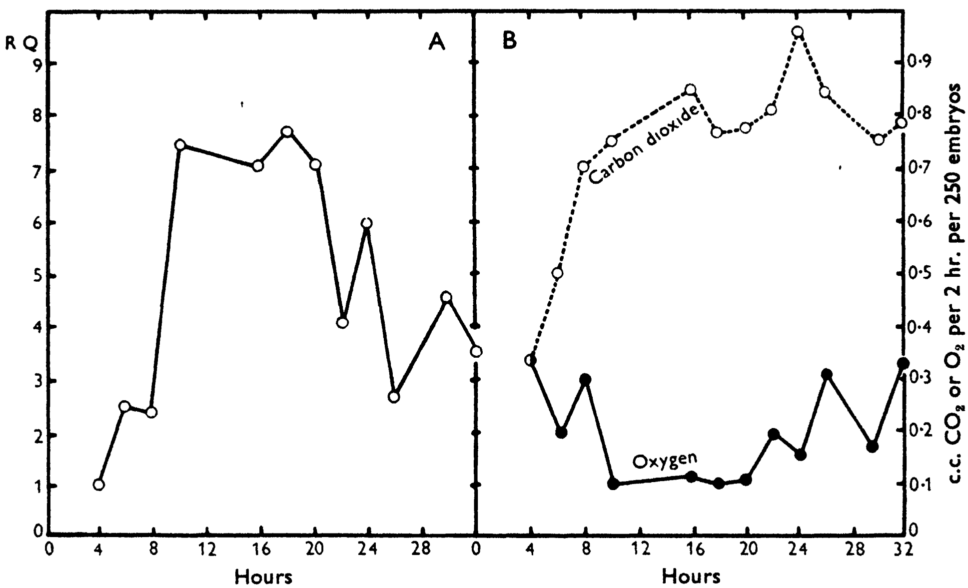
Oxygen uptake in the first phase

The rise of the R.Q. from the low value of the dormant grain to the steady value of 0·95 is not smooth. One large and other smaller oscillations are shown. These are not due to spasmodic releases of carbon dioxide, but to irregularities in the

* We are endebted to Dr J. R. O'Brien, of the Oxford Biochemistry Department, for carrying out these estimations for us.

rate of oxygen absorption. This is most clearly shown in Text-fig. 14, in which the oxygen and carbon-dioxide changes are plotted together. The carbon-dioxide emission rate (broken, heavy line) shows the usual steady acceleration without any considerable fluctuations. The thin line shows what the oxygen absorption would be if the R.Q. followed a regular transition from the dormant to the steady value of 0·95, as given by the thin line in Text-fig. 13. The actual oxygen absorption (continuous heavy line) shows instead two marked depressions which are clearly the sole causes of the simultaneous rises of the R.Q.

The origin of the oxygen block seems to be physical rather than chemical, and to be associated with the wetting of the seed. The low permeability of water to oxygen must materially increase the diffusion resistance if a film of water is placed



Text-fig. 15. Gas exchange of 250 isolated embryos immersed in shallow aerated water. A, the respiratory quotient. B, O---O CO_2 output, ●——● oxygen intake.

between the respiring cells and the surrounding air. This condition is exaggerated if the grains are immersed in a shallow dish of water instead of being pressed into moist sand.

An experiment was carried out in apparatus 4 in which 250 isolated embryos were immersed in a shallow capsule and the air stream blown through the contained water. The effect of the excess of water is shown (Text-fig. 15A) by a much increased rise in the R.Q., which is again due (Text-fig. 15B) to an oxygen lag. Although the CO_2 rate rises, the rate of oxygen absorption is actually lowered in this experiment for a time, and it is significant that, after 20 hr., it begins to recover and bring about a lowering of the R.Q.

Under the usual conditions, the oxygen block is only transient, and recovery is due in one form or another to the events of growth. The swelling of the colloids,

which is taking place rapidly during the first 24 hr., tends to increase permeability and may be important.

The block is probably general, rather than restricted to a single tissue or position. It is shown even in experiments with excised embryos (Text-fig. 12) with freely exposed scutellums. Further, removal of the concrescent glumes and paleae, which was habitually carried out in our experiments with whole grains, must inevitably have caused some damage to the surface, so it does not seem possible to ascribe any special effects to the fused pericarp and testa. A surface film of water penetrating to an unknown extent into the embryo seems to be the cause of the block.

Discussion

Factors influencing the gas exchanges of the first phase

The dormant grain absorbs oxygen more rapidly than it gives out carbon dioxide (R.Q. = 0·64). This inequality is due to the slow progress of incomplete oxidations over and above the oxidations leading to carbon dioxide formation. The ether-soluble material slowly diminishes during dormancy and its conversion is likely to be the most important of the incomplete oxidations. Amongst oily seeds, resting apple pips have been found to give an R.Q. = 0·7 (Harrington, 1923) and acorns have given R.Q.'s *circa* 0·5 at 20° C., falling to $<0\cdot1$ at 0° C. (Brown, 1939). There is nothing in our experiments against the simple assumption that the carbon dioxide lost is derived from the respiratory decomposition of carbohydrates with the absorption of an equal volume of oxygen. The rate of carbon dioxide output is very slow and can only be measured by enclosing a large quantity of grain in a confined space. A sample of air drawn from the centre of a mass of grain in an open bin showed no measurable increase of carbon dioxide.

The dormant grain normally contains about 14 % of moisture. Germination is initiated at any season when the grain is brought into contact with free water. The first contact with water has three consequences affecting the gaseous exchanges. A layer, probably often no more than a surface film, comes between the respiring tissues and the atmosphere. On account of its low permeability to oxygen, the water-film forms a slight oxygen block. In extreme cases, especially if the grain is totally immersed, the rate of entry of oxygen is actually decreased for a time; but more generally the check is expressed by a lag of oxygen consumption behind the rapidly rising carbon dioxide emission.

The second effect of the water becomes apparent as wetting of the tissues becomes more pronounced. Their semi-dry colloids swell, and in swelling become more permeable. It is, perhaps, on this account that the oxygen block disappears: that it does so is evidenced by a return of the R.Q. to a value a little higher than that of the resting grain. The very mild degree of anaerobiosis undergone meanwhile puts no restriction on germination.

The third effect of the approach of water to the grain is the quite sudden initiation of growth. Within the first 24 hr. appreciable breakdown of reserves, and measurable synthesis of cellulose and hemicellulose have taken place in the embryo.

Measurable material has also been received from the endosperm and there is every reason to believe that earlier analyses would still show similar changes occurring. There is no lag phase in the curve of carbon dioxide emission determined every 2 hr., but a diminishing acceleration from the very start (Text-fig. 7).

The early output of carbon dioxide is not likely to be due to a release of stored gas. When seeds that have been soaked under water (anaerobiosis) are brought into air to germinate, they often show a relatively fast rate of carbon dioxide release, which then slows down during the first few hours in air. Seeds which take up water from moist sand or cotton-wool have a slowly rising rate of emission from the first. The experiments of Geiger (1928) show both conditions with garden peas. Frietinger (1927) soaked wheat grains and found a falling rate of carbon-dioxide emission in the earliest stages after removal from the water. Our own barley grains were not soaked, and showed a rising rate from the start. It seems clear that carbon dioxide accumulates in the tissues during the soaking and is subsequently released; but if there is an accumulation prior to germination in unsoaked seeds it must be very slight.

The centre of this rapid acceleration of activity is the embryo: the endosperm shows a distinct lag at first. During the first 24 hr., starch decomposition was too small to be measured, but rose to 340 mg. per grain during the second (Hora, 1936). The starch-bearing cells of the barley endosperm appear to be almost incapable of spontaneous activity (Bruschi, 1908), and their evacuation depends mainly upon the secretion of enzymes by the scutellum (the evidence is summarized by Lehmann & Aichele). Our results show that, in the meantime, the development of the embryo proceeds at the expense of its own reserves, which therefore act as a metabolic priming, and include sucrose, raffinose and fats, but no starch. We can make the following rough account for the first 24 hr.:

Table 2. *Mg. hexose per* 100 *embryos: first day*

Formed		Broken down	
Cellulose	1·1	Raffinose	5·9
Hemicellulose	3·1	Sucrose	10·0
Maltose	4·4	Fats	(2·8)
	8·6		18·7

1 mg. fat assumed equivalent to 2 mg. hexose. The value given can only be very approximate and may be doubled or omitted without altering the conclusions drawn.

There is thus available 10·1 mg. for respiration and other syntheses. The total carbon dioxide emission of embryos plus endosperms was equivalent to 23·0 mg., but its partition between embryo and endosperm is uncertain.

Table 3. *Mg. hexose per* 100 *embryos: second day*

Formed		Broken down	
Cellulose	1·8	Sucrose	5·3
Hemicellulose	13·1	Raffinose	0·0
Maltose	4·4	Fats	(2·8)
	19·3		8·1

During the second day the position is completely changed. Raffinose is completely gone and the breakdown of sucrose and fats can no longer cover the increasing formation of polysaccharides. In addition, the respiratory consumption rose to 77 mg. The difference has to be met by gains from the endosperm, and a crisis occurs during the second day which is revealed as a check in the mounting rate of respiration (p. 161). Although the author makes no comment, a similar inflexion seems to exist in the records of Barnell (1937) for the variety Spratt Archer. In our detached embryos, which had no reserve to draw upon or only an external source of sucrose, there was an actual fall in the respiration rate.

The partial oxidation of fats detected in the resting grain continues throughout this phase. It is well known that during the first few days of germination of oily seeds, carbohydrates appear as fat is consumed and that, at the same time, the R.Q. falls as low as 0·3 while starch and cellulose are being formed at the expense of fat (summaries by Miller, 1938; Stiles & Leach, 1932). It is therefore reasonable to suggest that the oxidation of fat to carbohydrate (R.Q. = 0) is responsible for the depression of the R.Q. It does not seem to have been realized, however, that this explanation cannot be complete. In the results for linseed (quoted from Stiles & Leach) R.Q.'s below 0·5 persist at a time when analysis showed a net loss of carbohydrate (cellulose + starch). We must therefore suppose that the polysaccharides are further converted into "fibre" which evades the cellulose estimation or, more probably, that some other product of fat is also formed.

The discrepancy becomes much more striking in our barley embryos. When they develop their extremely low R.Q.'s, they are severely starved and under such conditions carbohydrates would not be expected to accumulate. In fact, the analyses of the preceding paper show that the carbohydrates present are rapidly used up, and there is no net increase at any stage. We therefore turned to the other known product of fat oxidation, the cutins. These may be regarded as complex condensation and oxidation products of fatty acids (Priestley, 1924); and though they continue to dissolve fat-soluble dyes, they are no longer themselves soluble in ether. As a result of a variety of tests it was shown that the coleoptile and the first leaf rolled within it both had a well-developed cuticle (p. 168). Since these organs increase their surface very rapidly by cell enlargement in the early stages of germination, there can be little doubt that cutin is being produced. A quantitative comparison of fat consumption and cutin formation is not practicable, but a partial oxidation of fat accompanied by cutin formation and an excess oxygen consumption seems to be demonstrated. It seems likely to contribute towards the depressed R.Q. of fatty seeds also.

Phase 2. Mobilization of endospermic carbohydrate

Respiration rate in this phase increases because the respiratory machinery is growing, both in the embryo and in the endosperm. There is, for example, a rapid increase in the activity of dextrinogenic amylase and invertase at this time. As a result of the amylase activity, starch is rapidly dissolved and soluble products, "dextrins", accumulate in the endosperm (Hora, 1936). The acceleration of respira-

tion is, however, held in check (it is no longer a free exponential increase) by the rate at which carbohydrate from the endosperm is made available in the embryo. CO_2 output from the endosperm itself (barley, var. Spratt Archer) is estimated by Barnell (1937) as only 10 % of the whole respiration, during a period corresponding with our first two phases. It remains uncertain whether the restricting rate is that of translocation or of a chemical conversion such as dextrin to sucrose.

The limit to this phase will thus be set when the rate of carbohydrate supply can no longer meet the demands of synthesis and respiration in the embryo. It corresponds fairly closely with the time at which starch can no longer be detected in the endosperm, and the dextrins reach their maximal concentration. Sucrose also reaches its highest concentration in the embryo at the same time (A. L. James) and respiration rate its peak; but a gain of cellulose and hemicellulose ("synthesis") continues into the next phase.

Although the ultimate substrate of respiration is, in the main, the starch of the endosperm, the active embryonic cells contain little starch, but an amount of sucrose which increases throughout this phase. It seems probable that this is the immediate substrate of respiration in the embryo, as it more certainly is in the phase before, and the phase after. The R.Q. approaches unity with a value of 0·94 in 1934 seed and 0·95 in later experiments not fully reported here. In the 1933 grain (Text-fig. 12) it was lower, and continued to rise towards 0·95, suggesting that the oxidation of fat evident in the first phase may also continue into this one. Since the slight depression of the steady value below 1 is beyond the experimental error and shown in every experiment performed, it may have a similar interpretation.

In embryos isolated from their endosperms, this second phase is much less pronounced. It opens with a falling respiration rate (Text-fig. 9) and feeding with an external supply of sucrose cannot do more than maintain the rate at the end of phase 1, in place of the normal fourfold increase of phase 2. The study of the R.Q. (Text-fig. 12) suggests that the rise which follows is to be compared with phase 4 of the normal seedlings (*q.v.*) rather than phase 2.

Phase 3. *Exhaustion of endospermic carbohydrate*

The falling respiration rate of this phase coincides with a complete absence of starch and a falling concentration of dextrins in the endosperm. In the embryo itself, sucrose begins to diminish again, and though hemicelluloses continue to be synthesized at first, they reach their maximum before the phase is closed. These carbohydrates are the whole, or almost the whole, source of respired material, since the R.Q. remains steady at its maximal value, just below 1.

It is noteworthy that, under the conditions of mild starvation of phases 1 and 3, sucrose and raffinose are both rapidly consumed. These two sugars are fructofuranosides and their behaviour is in marked contrast with that of the pyranoses and pyranosans at the same time. Total hexose, maltose, hemicellulose and cellulose all accumulate. This is evidence for the view already put forward (James, 1938) that barley respiration breaks down fructofuranose (γ-fructose) most readily, though not to the complete exclusion of pyranose.

Phase 4. Breakdown of non-carbohydrate materials

The carbohydrate content of the endosperm is reduced to a very low ebb, and dextrins, like starch, are no longer detectable. In the embryo itself, not only is sucrose reduced to a low concentration, but the pyranose anhydrides (maltose and hemicellulose) are also diminished, as well as the pyranoses themselves. The fall of respiration rate is nevertheless arrested to some extent, and although it continues, does so slowly (Text-fig. 5). This suggests the breakdown of some additional non-carbohydrate material side by side with the consumption of the residual carbohydrate. The R.Q. begins to fall, and drops to nearly 0·8 by the end of the phase, suggesting that the new substrate may be protein. This phase would thus be analogous with the better known "respiration hump" of detached leaves, which causes a similar arrest in the falling respiration rate, or even a temporary increase. Such an increase is also evident in the respiration drifts of detached embryos (Text-fig. 9), even when artificially fed with sucrose. The embryos without such feeding contain only a minimum of utilizable carbohydrate at this stage, as shown by the analyses of the preceding paper, and their R.Q. varies around 0·5. Such an R.Q. seems to be characteristic of extreme starvation and dependence upon protein, although much lower than the R.Q. (0·7–0·8) to be expected from simple protein oxidation. It also occurs in the seedlings from normal grains at a much later stage where it is probably due to putrefaction, i.e. protein breakdown by saprophytes. Fungi on protein media are said to give R.Q. 0·5 (Stiles & Leach, 1932). The relatively high R.Q. of the normal seedlings in phase 4 attests the persistence of a carbohydrate contribution to respiration; just as it is raised by artificial sugar feeding. By the end of the phase there is a virtual exhaustion of all soluble carbohydrate, and the respiration slowly declines until the final collapse of the cytoplasmic structure leads to the post-mortem respiration of phase 5.

SUMMARY

1. Apparatus is described which has been used for a number of investigations on the respiration of barley.

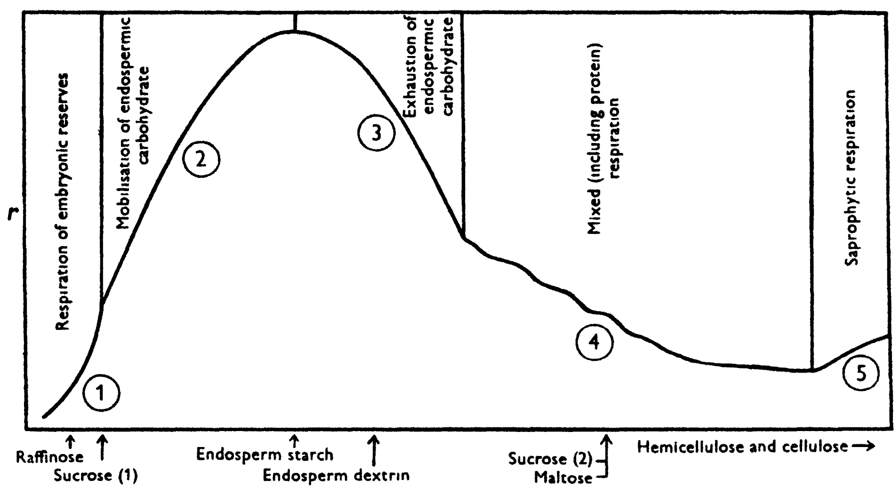
2. Continuous records of the CO_2 emission of grains germinating in the dark yield a curve which is analysed into 5 phases (Text-fig. 16). Each phase is characterized by a special factor which becomes dominant at its particular stage and recedes to a relatively small importance at other stages. These factors are, in succession: (1) free embryonic development; (2) mobilization of endospermic carbohydrate; (3) exhaustion of these reserves; (4) breakdown of other (probably protein) materials; (5) saprophytic respiration.

3. Embryos germinated independently of their endosperms show a similar first phase and much modified later stages. Artificial feeding with sucrose only very partially compensates for the absence of the endosperm.

4. The drift of the R.Q. of seedlings germinated in the normal way with their endosperms is more complicated than reported by previous workers (Text-figs. 12, 13). In the absence of endosperm the R.Q. followed a more or less parallel but lower course (Text-fig. 12). Without either endosperm or artificial feeding it fell for a time below 0·3. The R.Q. of the dormant grain was 0·64.

5. The ether-soluble material in 100 grains was determined before and after an interval of 15 weeks; and was found to have diminished. It was also reduced after 50 hr. germination, i.e. after the first respiration phase and when the R.Q. was very low.

6. Cutin was formed over the surfaces of the rapidly developing coleoptile and first leaf rolled within it. The low R.Q. of the isolated embryos was not associated with carbohydrate formation.



Text-fig. 16. The respiration drift of barley seedlings germinating in the dark. Diagrammatic, based on Text-fig. 5 and similar records. The phases 1–5, and the evidence on which they are based, are described in the text. The arrows show, approximately, the stage at which the substances named are exhausted. Except for starch and dextrin they refer to the embryo seedling proper.

From a consideration of the data of this and the preceding paper, we suggest the following outline of events determining the drift of respiration.

The very slow respiration of dormancy is accompanied by an equally slow partial oxidation of fatty materials, probably leading to the formation of cutin. In *phase* 1 the application of water immediately touches off a rapid development of the embryo; which is sustained mainly by sucrose and raffinose stored in the embryo itself. These give rise to "hemicelluloses" with a trace of starch, and true cellulose as well as the CO_2 of respiration. The partial oxidation of fats continues throughout this phase and the next. It gives rise to cutin and, less probably, to carbohydrates accumulated as fibre. There is a transient restriction of oxygen uptake, probably owing to the wetting of the grain, which has a very striking effect in raising the R.Q., but little influence on respiration and development. *Phase* 2: The reserves of the embryo itself being exhausted, development and respiration now depend on the rate of supply from the endosperm. This involves the conversion of starch to dextrins and of dextrins to sucrose, as well as the actual transport of carbohydrate from endosperm to embryo. Although rapid solution of starch in the endosperm is the ultimate source of material, the immediate substrate of respiration in the embryo is probably sucrose whose concentration rises like the respiration

rate. *Phase* 3: At about the same time that starch finally disappears from the endosperm, the respiration rate begins to decline. The endosperm shows a falling concentration of dextrins, and in the embryo sucrose is again diminishing. Towards the end of the phase, hemicelluloses begin to disappear also. These carbohydrates are the entire, or almost entire, source of respiratory material, since the R.Q. is only slightly below 1. It is noteworthy that, under the conditions of mild starvation of phases 1 and 3, sucrose and raffinose (both fructofuranosides) are rapidly consumed, while maltose, hemicellulose and cellulose (all pyranose anhydrides), and the pyranoses themselves, accumulate. This supports the view that, in barley, fructofuranose (γ-fructose) is respired more readily than—though not to the exclusion of—pyranoses. *Phase* 4: The endosperm being now virtually exhausted of reserve carbohydrates, the check in the fall of respiration rate suggests the utilization of some new substrate. This phase is analogous with the better known "respiration hump" of starving leaves, and the new substrate is likely to be protein. In confirmation, the R.Q. sinks to 0·8 by the end of the phase.

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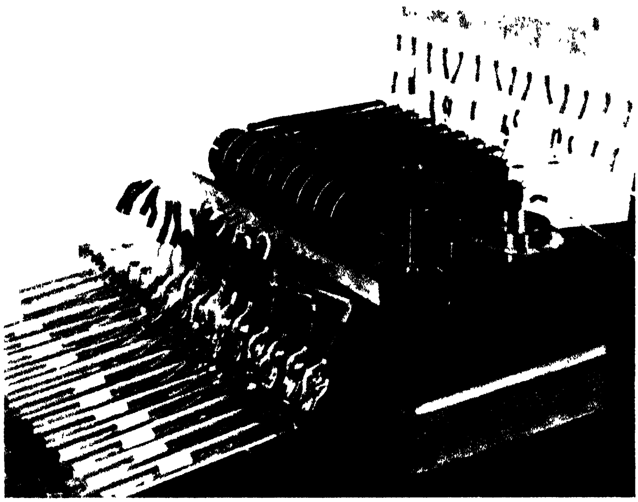
EXPLANATION OF PLATE 3

A. Apparatus 2, automatic gas switch, for description see p. 146.

B. Contents of thermostat, i.e. plant chambers, etc., used for first germination experiments (see p. 155).

C. Embryos dissected from their endosperms and germinated on sand moistened with culture solution and 6 % sucrose; 5 days old. × 0·7. The coleoptiles are translucent, and the opaque first leaves can be seen developing inside. Small roots can be seen where they have not penetrated the sand. Abundant tufts of root hairs develop near the embryos themselves.



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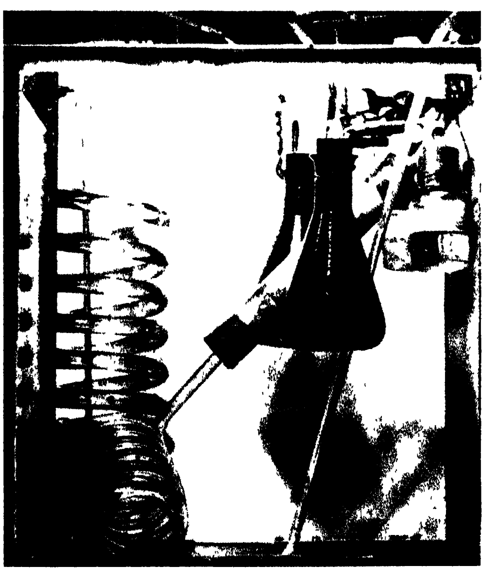

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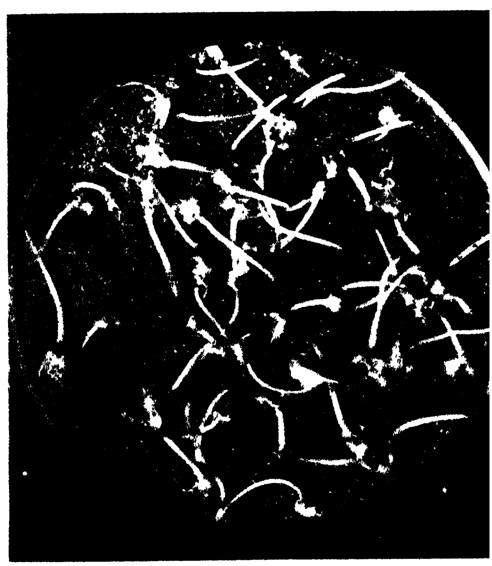

C

JAMES AND JAMES—THE RESPIRATION OF BARLEY GERMINATING IN THE DARK

A HORMONE FOR CORRELATIVE INHIBITION

By R. SNOW
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(With 1 figure in the text)

1. Introduction

From various experiments the writer concluded previously (1937, 1938) that the inhibition of lateral buds and shoots by auxin, whether formed naturally or applied, is brought about indirectly and is due to a secondary inhibiting influence, which originates from some primary process promoted by the auxin in the main stem and travels upwards into lateral buds and shoots where the auxin cannot easily follow it. As to the nature of this inhibiting influence, an earlier experiment (Snow, 1929) indicated that it was probably an inhibiting substance, since inhibition could travel upwards through a dead zone with the transpiration stream. But for reasons given previously (1937, p. 298) it seemed desirable to obtain further evidence on this point, and an experiment providing such evidence will be reported here. This experiment, like others reported previously (1937, p. 292; 1938, p. 180) provides evidence against Went's "diversion" theory of inhibition also (1938, 1939), which will be discussed again in section 3.

2. Experiment

Peas, of race "Thomas Laxton", were sown in pairs close together, and the main shoots were cut off as soon as they appeared. Later when the axillary shoots of the cotyledons, two from each plant, had grown out and had fully expanded two leaves, those pairs of plants were selected in which at least three of the four cotyledonary shoots were well developed, and the fourth shoot was removed, so that in each pair of plants there now remained one plant with two shoots and another with one (see Fig. 1, for which I am indebted to my wife). Then one of the shoots of the "two-shoot" plant was tied with moist bast to the single shoot of the other plant, in such a way that the internodes second from the base were pressed fairly tightly into contact along most of their length. Before these internodes were tied together, thin strips of epidermis and a little cortex had been cut away from the sides which were to be brought into contact, and the cut surfaces had been washed. Care had been taken to select shoots in which these internodes would fit together easily and fairly exactly. Finally, the shoots which had been tied together were decapitated by cuts through the next internodes above those which had been tied. The object was now to note whether the intact shoot of the "two-shoot" plant would inhibit the lower axillary bud of the single shoot of the other plant beyond and below the region of contact. The controls were the corresponding buds of similar shoots which were decapitated at the same level but not bound to neighbouring plants.

The buds to be observed were from 0·5 to 1·0 mm. long at the start, and care was taken that those of experiments and controls were about equal in length. Shoots with larger buds were avoided. After the binding and final decapitation, the plants, grown in the summer in a green-house, were kept in the shade in rather

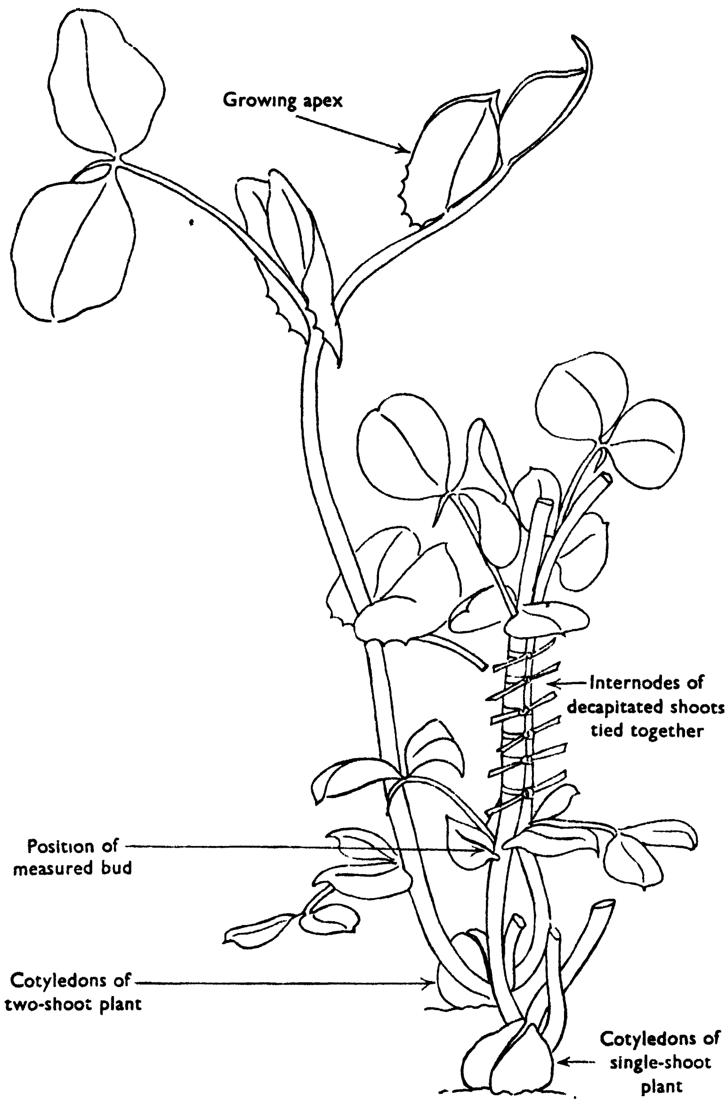

Fig. 1.

damp air. The buds of experiments and controls were measured 6 days after the final decapitation in the first batch of plants, and 5 days after it in the others. Table 1 shows the growth of the measured buds after these times.

It can be seen that the mean growth of the buds at the lower nodes in the "single-shoot" experimental plants was less than half as much as in the controls.

Moreover the greatest bud growth in the six experimental plants was less than the smallest bud growth in the sixteen controls. Clearly therefore the buds of the experimental plants were partially inhibited by the intact shoots of the other plants across the region of contact. The buds at the next higher node also, above the region of contact, grew distinctly less than the corresponding buds in the controls, but this will not be considered as evidence since it might be argued that they were retarded by the tightness of the ties round the stems below them. All the lateral buds on the "two-shoot" plants, including those on their decapitated shoots, were of course completely inhibited.

Table 1. *Growth of measured buds after 5 or 6 days*

Batch 1		Batch 2		Batch 3	
Experimental plants mm.	Controls mm.	Experimental plants mm.	Controls mm.	Experimental plants mm.	Controls mm.
0·75	6	4·75	8	3·25	6
3·25	6	4·0	9	4·5	8
	10		6		7
	8		6		11
	8		6		14
					16

Means: Experimental plants 3·82
Controls 8·44

After the experiment the bast ties were cut through and the stems fell apart at once, showing that they had not adhered nor grown together. So the inhibiting influence which travels up a side shoot can cross a moist protoplasmic discontinuity, and this result, together with the previous "dead-zone" experiment (1929), justifies the conclusion that the inhibiting influence is another hormone. Previous experiments (Snow, 1939*a*) indicated that the inhibiting influence originates from the reaction or co-operation of auxin with some other factor or factors in the main stem.

3. The "diversion" theory of inhibition

Went (1939) has recently reported some experiments which he claims to support his "diversion" theory of inhibition by auxin, and these will be discussed below. According to this theory, the auxin, whether formed naturally by the apical bud or applied artificially, brings about the inhibition of lateral buds by somehow causing some substance necessary for the growth of buds to travel up the main stem to the apical bud instead of passing out into the lateral buds. Thus lateral buds (and presumably lateral shoots) remain cut off from their supplies at the base, which are diverted up the main stem. The writer has previously reported two experiments on correlative inhibition (1937, p. 292) and one on inhibition by applied auxin (1938, p. 180) which cannot be explained on this theory, besides another experiment (1937, p. 293) which would be difficult to explain on it; and Went has not attempted to answer these objections. The experiment reported in the previous section is also

impossible to explain on Went's theory, as a glance at the illustration will soon show. For the auxin travelling down the main stem of the "two-shoot" plant cannot cut off the lateral buds of the other plant from their base of supplies, since they have their own independent base which that auxin cannot reach. Indeed the arrangement is physiologically rather similar to that in the writer's previous experiment (1937, Figs. 2, 3), though differently reached.

The earlier "dead-zone" experiment (Snow, 1929) is also quite inconsistent with the "diversion" theory. For it was found that if a zone of stem was killed near the base of a decapitated shoot of a "two-shoot" broad bean plant, the buds above the dead zone grew out a little, though they grew only very slowly since some inhibition from the other shoot travelled up to them through the dead zone. But still they did grow a little, whereas in plants similarly prepared, but without a dead zone, the corresponding buds did not grow at all. Thus by killing a zone of stem on a decapitated shoot, one releases the buds above the zone partially, though not completely, from inhibition by another shoot. Yet one certainly does not improve their chances of drawing supplies from the base.

Went (1939), by applying hetero-auxin in lanoline to decapitated dark-grown pea shoots, has confirmed the "increase of inhibition with distance" which was previously found by the writer (1931) using part of the shoot apex as the source of inhibition. This confirmation is welcome, and Went argues that it can be readily explained on his "diversion" theory since it may be supposed that for effective inhibition a sufficient length of stem is needed in which the factors for bud growth may accumulate above the lateral bud. This explanation is indeed plausible in itself, but Went leaves out of account the fact that he himself (1939, p. 110) found that seven other chemicals ("auxinoids", as they may perhaps be called), which have some of the effects of auxin and hetero-auxin but are less rapidly transported, caused not an increase but a decrease of inhibition with distance. The conclusion should surely be that the "increase with distance", though still unexplained, is probably, when it occurs, somehow connected with the high mobility of natural auxin and hetero-auxin. It may be mentioned that Thimann (1937), using dark-grown pea shoots and hetero-auxin, found neither significant increase nor decrease with distances ranging from 2 to 150 mm.

Went also claims to show by some rather complicated experiments (1939, Tables 3, 4, 5) that in shoots of decapitated etiolated pea seedlings certain rather weak concentrations of hetero-auxin and of γ-phenyl butyric acid in lanoline, applied on top of the stem, both inhibit lateral buds and also increase the accumulation of factors for bud growth in the stem after the decapitation. (Even when pure lanoline was applied, the factors for bud growth accumulated similarly in the stem, if Went's interpretation is correct, but to a somewhat less degree.) He claims that the results support his theory that inhibition of lateral buds is due to diversion of factors for bud growth to the top of the stem. But actually, as Thimann (1939, p. 337) has pointed out, in these experiments these concentrations of the applied chemicals did not inhibit the lateral buds, but promoted them, although they did inhibit the lateral buds in other conditions, when the shoots were left on the plants

instead of being used as cuttings (Went, 1939, p. 114 and Table 7). But then in those conditions there was no evidence of increased accumulation of bud growth factors in the main stem.

Even apart from this objection, and even if Went's interpretation of these experiments were entirely granted, it would only have been shown that, after the substances mentioned have been applied, two results follow; the factors for bud growth accumulate in the stem, and the lateral buds are inhibited. From this it would not follow that one of these two results is the cause of the other.

Went also reports (p. 116) that some low concentration of hetero-auxin inhibits the lateral buds if applied continuously after decapitation, but promotes them if applied for the first 2 days only: and he claims that this fact is "completely unexplainable on the basis of Snow's theory". But this is not so, for at least one possible explanation can readily be suggested, which is not inconsistent with that theory. Thus application of hetero-auxin for 2 days only may perhaps be equivalent to application of a very small continuous dose. If so, then the explanation may be that a very small dose of hetero-auxin, leading, according to the writer's theory, to the formation of a very small amount of inhibitor, may act on the lateral buds in the opposite way to a larger dose; just as hetero-auxin promotes root growth in very low concentration, but inhibits it in higher concentration. However, the true explanation may of course really be quite different.

4. The direct and indirect theories of inhibition

The indirect theory would be much strengthened if an inhibitor could be extracted from those parts, and only those, in which it would be expected. Recently, several workers have obtained extracts containing inhibitors of coleoptile growth. Thus Viehmann (1939) has obtained by diffusion from sunflower hypocotyls a growth inhibitor which, as he concludes, is formed either in the light, or else in the dark, but then only under the influence of applied acetic or formic acid. Stewart and others (1939) and Stewart (1939) have extracted with ether from radish cotyledons an inhibitor which is transported in both directions. Larsen (1939) has extracted inhibitors with ether from tomato fruits and from meal of maize seeds, and Goodwin (1939) has concluded from indirect evidence that ether extracts of maize meal and of broad bean shoots contain an inhibitor. The writer (1939*b*), following Stewart and others, has extracted a rather different inhibitor with wet ether from pea leaves, but this inhibitor was found sometimes to persist for several days at least in the leaves of decapitated disbudded shoots, and not to disappear as the correlative inhibitor would be expected to do. The same test would need to be applied to the other inhibitors also, before they could be accepted as likely candidates for the position of correlative inhibitor.

In a valuable review Thimann (1939) considers the "indirect" theory of inhibition as still remaining open, but raises some difficulties for it, which to the writer do not seem insuperable. Thus in referring (p. 327) to his experiments in which he inhibited buds by applying auxin in lanoline to their outer sides (1937),

he does not take into account the fact that in such experiments the auxin travels down the outer leaves and enters the axis of the bud from its base. Consequently the inhibition can be understood on the indirect theory.

Thimann objects also (p. 332) that the indirect theory does not explain the great decrease of auxin in inhibited parts. But it is to be expected that when these parts are inhibited in growth, they will cease to form auxin, as seems probably to have happened in the seedlings of Avery *et al.* (1937) when grown with shortage of nitrogen. Or again, if the suppression of auxin formation in inhibited buds and shoots is primary and the cessation of growth secondary, then the correlative inhibitor may act by suppressing the auxin formation. In spite of what Thimann writes on this point, the essential difference from his "direct" theory would still remain, that on that theory the auxin from the inhibiting region must travel into all inhibited lateral buds and shoots, whereas on the "indirect" theory it does not do so, or not in any effective concentration. Indeed on the "indirect" theory those parts of the shoot system which are so situated and orientated that the main stream of auxin, travelling chiefly downwards, cannot easily enter them are just those which become inhibited. Evidence that this is indeed the rule was offered previously (Le Fanu, 1936; Snow, 1937, 1938). It is not necessary on this theory to suppose that *absolutely* no auxin enters the lateral buds and shoots; for it is enough if the ratio of auxin to inhibitor is much lower in them than in the main stem. Indeed van Overbeek (1938, p. 160) has obtained evidence of some slight upward transport of hetero-auxin into lateral buds growing out in the dark.

It would, however, undoubtedly be difficult to explain, on the indirect theory, the inhibition of the formation of adventitious buds in flax hypocotyls by hetero-auxin, or by ether extracts of shoots, applied in lanoline on top (Link & Eggers, 1938), or again, as Thimann has pointed out, to explain the inhibition of regeneration in fern prothalli by hetero-auxin in lanoline applied to the apical cut surface (Albaum, 1938). For in these examples the inhibited centres of regeneration are not inserted laterally, so that the downward-moving auxin probably passes directly through them. Contrasting indeed with these two examples are several others in which the formation of adventitious buds from stems or leaves in various species has actually been provoked by hetero-auxin in lanoline applied from above (Greenleaf, 1937; Beal, 1937; Goldberg, 1938; Prevot, 1938). Nevertheless it may be pointed out that it would be quite consistent to explain on the basis of Thimann's "direct" theory such examples as the inhibition of regeneration in flax hypocotyls and fern prothalli, while still explaining on the "indirect" theory the inhibition of lateral buds and shoots, for which alone it was intended.

On this combined view the fate of any part of the shoot system depends on two variables, the relative amounts of auxin and inhibitor which it receives, and its inherent sensitivity. Thus some parts, such as lateral buds and shoots, are so situated and orientated that the main stream of auxin, travelling chiefly downwards, cannot enter them or only in small amount; and these parts are regularly inhibited by the inhibitor whatever their stage of development, in accordance with the "indirect" theory. But those parts through which the descending stream of auxin

directly passes react to it in a manner depending on their inherent sensitivity, as proposed by Thimann (1937). For instance stems generally have their growth in thickness promoted by moderate concentrations of auxin passing down them, and their growth in length either increased or, if they have enough auxin already, not much affected by it. Also growth in buds is apparently promoted by moderate amounts of auxin when it is really applied to their morphological apices, and not to their outer leaves: for hetero-auxin in lanoline applied to part of the stem apex was found to promote the formation and growth of members from that part (Snow & Snow, 1937). (Thimann (1939) has a little misunderstood these experiments: the auxin was applied to the stem apex or to part of it, not to the existing leaf primordia.) But other parts, such as the centres of incipient regeneration in flax hypocotyls and fern prothalli, may be more sensitive to auxin passing through them, so that unless the concentration of auxin is low, it may be too much for them and inhibit them. This explanation agrees well with experiments of Stoughton & Plant (1938) also, which indicate that in sea-kale cuttings a high content of auxin tends to inhibit formation of adventitious buds.

The very earliest stages of formation of axillary buds can also be shown to be liable to correlative inhibition, in peas at least, if the plants are given the opportunity to form new buds by regeneration in one of the lower axils (Snow, 1931); and for the present it must be left an open question whether this inhibition is direct or indirect. But further evidence that from quite an early stage of development the axillary buds are inhibited indirectly is the fact that they can quite early be shown to be negatively geotropic, even while partially inhibited.

To test this point, the writer bent down long shoots of pea seedlings horizontally and reduced the inhibiting power of the apex either by removing the older developing leaves, or else by decapitating high up and leaving a single developing leaf (see Snow, 1931). Controls were decapitated and deprived of all developing leaves. The buds of the plants with apex or single young leaf remaining grew out much the more slowly, being partially inhibited; but yet they showed clear geo-negative curves in their basal internodes when these internodes were only from 4 to 8 mm. long, and the whole buds from 8 to 13 mm. long. Their negative geotropism indicated that auxin was promoting their growth, while at the same time they were being correlatively inhibited. The inhibition therefore can hardly have been directly due to auxin. In general, if axillary buds were inhibited directly by auxin, one would expect often to notice them making geo-positive curves in nature.

SUMMARY

1. An experiment illustrated in Fig. 1 shows that in pea plants correlative inhibition from a growing shoot apex can travel down one shoot and up another decapitated shoot, and can then enter a decapitated shoot of another plant, crossing a protoplasmic discontinuity where the tissues are only in moist contact.

2. Since previous experiments had shown that the inhibiting influence which travels up a lateral shoot is not ordinary auxin (Snow, 1937), it is concluded

from this experiment together with an earlier one (Snow, 1929) that the inhibiting influence is another hormone.

3. The above experiment, like others reported previously (Snow, 1937, 1938), cannot possibly be explained by Went's "diversion" theory of inhibition (1938). Some recent experiments, which Went (1939) claims to support his theory, are discussed and considered inconclusive.

4. Some difficulties raised by Thimann (1939), against the "indirect" theory of inhibition in shoots, are discussed, and it is pointed out that the inhibition by auxin of the formation of adventitious buds in the main axis may be direct, even if the inhibition of lateral buds and shoots is indirect. Further evidence that the inhibition of lateral buds is not directly due to auxin is provided by an experiment showing that in peas they are negatively geotropic even while partially inhibited.

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FRUCTOSANS IN THE MONOCOTYLEDONS. A REVIEW

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(With 8 figures in the text)

CONTENTS

1. Historical introduction

The study of the naturally occurring polymers of fructose began in 1805 with the discovery of inulin in the root of elecampane (*Inula Helenium*). Later Tanret (1893 *a*, *b*, *c*) claimed to have isolated four more fructose polymers (pseudo-inulin, synanthrin, helianthenin and inulenin) accompanying inulin in the artichoke (*Helianthus tuberosus*). It is now generally accepted that inulin is frequently accompanied by other fructose polymers (*Ann. Rep. Chem. Soc.* 1931), but it is doubtful whether the four substances described by Tanret were pure compounds, they may have been mixtures of such polymers (Thaysen *et al.* 1929). Inulin and its analogues are found chiefly if not exclusively in the Compositae (Colin, 1925). During the period 1870–1900 a number of fructose polymers was discovered in the monocotyledons. These were soon distinguished from the inulin group, mainly on account of their great solubility in cold water. While the term fructosan should properly apply to the whole group of fructose polymers including inulin, it is frequently used to designate only this second class of polymers found in monocotyledons, and it is in this restricted sense that the term is used here.

Among the early isolations of fructosans are those of Ludwig & Müller (1872, 1873) from the rhizomes of *Agropyron repens* (*Triticum repens*) and of Schmiedeberg (1879) from the bulbs of *Scilla maritima* (*Urginea scilla*). These plants had long attracted interest owing to their use in pharmacology, and it appears that the carbohydrates later identified as fructosans had previously been confused with the gums and pectins. The presence of fructosans in barley and rye was reported by Kühnemann (1875) and Müntz (1878, 1886). Their discovery in cereals resulted from the

attempts of agricultural chemists to isolate dextrins as intermediate products between sugar and starch during the development of the grain. Dextrins were well known at this time as products of starch disintegration *in vitro*, and this knowledge led to a prolonged search for these compounds in the living plant. A number of more or less impure carbohydrates were prepared, some of which were called dextrin solely on account of their solubility in water and lack of copper reducing power. Where polarimetric observations were made, however, it was found that many of these products were *l*-rotatory and could not therefore be dextrins. Such *l*-rotatory substances were found in rhizomes of *Iris pseudacorus* (Wallach, 1888), in *Allium sativum*, *Asphodelus microcarpus*, and *Polianthes tuberosa* (Chevastelon, 1894), in a number of grass rhizomes (Ekstrand & Johannson, 1887, 1888; Schulze, 1899; Harlay, 1901), in barley plants (Kjeldahl, 1881), stems of rye (Schulze & Frankfort, 1894; Schulze & Frankfort, 1895), in the unripe grains of barley, wheat and rye (Tanret, 1891) and in the leaves of *Yucca filamentosa* (Meyer, 1885). Some of these early preparations were not very pure, the most usual impurity being sucrose. The immediate development of fructosan chemistry following on these isolations appears to have been delayed by the prolonged controversy which arose as to the presence or absence of dextrins. During this time the significance of these *l*-rotatory fructosans was overlooked and no further progress was made for 20 years, when interest was revived in France and later in Germany. The more modern work has confirmed the presence of fructosans in the plants examined by the early workers (Augem, 1928; Belval, 1924, 1933, 1939; Colin & Augem, 1927; Colin & Belval, 1922, 1923*b*, 1934, 1937; Colin & Chaudun, 1933; Colin & de Cugnac, 1926; Colin & Neyron, 1927, 1931; de Cugnac, 1931*b*; de Cugnac & Belval, 1939; Kihara, 1935*a*; Kizel & Keretovisch, 1934; Norman, 1936; Schlubach *et al.* 1929–37; Tillmans, 1928), and added a few more to the list (Belval, 1933, 1937; Colin & Belval, 1937; Colin & Chaudun, 1933; Challinor *et al.* 1934*b*; Kylin, 1918; Tanret, G. 1909). In addition, Colin and his collaborators have studied the distribution of fructosans in cereals, in grasses, in *Iris*, *Lycoris*, and *Asphodelus*, and discuss their significance in the general carbohydrate metabolism of the plants, and the relationships of the several fructosans to those found in other plants. Finally, Archbold (1938) and Barnell (1938) have investigated the distribution and seasonal fluctuations of fructosans in barley and wheat respectively, while Russell (1938) has studied the effects of various mineral deficiencies on fructosan metabolism in barley.

In the early history of fructosan chemistry great stress was laid on the occurrence of these compounds in storage organs and their reserve functions, although their existence in leaves (Meyer, 1885; Müntz, 1886) and stems (Schulze, 1899) was observed at the outset. While recent work supports the view that large amounts of fructosan may occur in temporary reserves of carbohydrate in bulbs, and immature grain, they are now known to occur in smaller amounts in the leaves of *Iris foetidissima* (Augem, 1928), *Scilla nutans* (Colin, 1925), *Lycoris radiata* (Belval, 1933), *L. squamigera* (Belval, 1933), and of rye grass, Western Wolths (Norman, 1936), *Poa trivialis* (Challinor *et al.* 1934*b*) and barley (Archbold & Barter, 1935) as well as in the stems of cereals (Colin & Belval, 1934; Archbold, 1938; Barnell, 1938), and

of several species of *Elymus* (Belval, 1938). It seems likely therefore that fructosans are distributed throughout the plants in which they occur, though the amounts found in leaves may be very small, and at some stages in the life cycle they may be absent from leaves altogether. This distribution throughout the plant, coupled with the solubility in water, makes it desirable that in studies of carbohydrate metabolism changes in fructosan content should be considered principally in relation to changes in the simple sugars (Belval, 1924).

2. Occurrence

The recorded occurrence of fructosans in the monocotyledons, together with the specific rotations of isolated products, is given in Table 1. In some instances the fructosan was not isolated but its presence deduced from the results of sugar analysis, either polarimetric or by copper reduction, after hydrolysis of the plant extract under appropriate conditions. These cases are marked with a star in the table. In general, the names given to the isolated products are derived either from the plant name (e.g. irisin, scillin) or refer to the *l*-rotatory properties of the fructosan (e.g. sinistrin, lévosine). The names used by the various investigators are included in Table 1, but the nomenclature is far from satisfactory. Thus the fructosan obtained from *Scilla maritima* is known both as scillin and sinistrin, while those from *Arrhenatherum bulbosum* and from rye, which are probably different substances, are both named graminin. Two further names, secalose and lévosine, have also been used for the fructosan of rye. The exact constitution of the fructosans is still a matter for research, and at present it appears that the majority of the known compounds differ slightly in structure one from another. It is therefore premature to attempt to place the nomenclature on a more satisfactory basis, and the use of a general term such as fructosan together with the name of the plant under investigation is less confusing than that of a name which may have been applied to fructosans from several sources.

It is of some interest that fructosans may result from the activity of bacteria (*B. mesentericus*, *B. subtilis*) (Hibbert *et al.* 1931; Hibbert & Brauns, 1931; Veibel, 1938), and of mould spores (Kopeloff *et al.* 1920) when these organisms are supplied with sucrose. There is evidence that the substance produced by the activity of *B. mesentericus* is very similar to, if not identical with, the fructosans of *Poa trivialis*, and of the barley leaf (Challinor *et al.* 1934*b*; Haworth *et al.* 1937). The amounts of fructosan found in the several plants vary within rather wide limits and are of course subject to marked seasonal changes (see pp. 204–210) as well as to the other environmental conditions (see pp. 212–216). A selection of results taken from the French work is given in Table 2 to illustrate the order of the values found. Where possible the maximum and minimum recorded values are reproduced.

3. Preparation of pure samples

All the methods of preparation which have been described depend upon the fact that fructosans are soluble in cold water but not in alcohol. The process of their extraction from plants therefore consists essentially in macerating the tissues with

Table 1. *Occurrence of fructosans in plants (excluding inulins)*

Plant	Name given to fructosan	Isolated by	Year	Specific rotation $\alpha_D^{20°}$
Scilla maritima (bulbs)	Sinistrin	Schmiedeberg	1879	−41·4
S. maritima (bulbs)	Scillin	Riche & Rémont	1880	−44
S. maritima (bulbs)	Sinistrin	Reidemeister	1880	−34·6
S. maritima (bulbs)	Sinistrin A and Sinistrin B	Schlubach & Flörsheim	1929	−25·3 −36·6
S. maritima (bulbs)	Sinistrin B	Schlubach & Loop	1936	−44·5
S. maritima (bulbs)	Scillin	Colin & Chaudun	1933	−27
	Scillin			−43·7
S. nutans	"Fructoside"	†Colin & Chaudun	1933	
Iris pseudacorus (rhizomes)	Irisin	Wallach	1888	−52·3
I. pseudacorus (rhizomes)	Irisin	Augem	1928	−51·5
I. pseudacorus (rhizomes)	Irisin	Schlubach *et al.*	1933	−54·7
I. foetidissima	—	*Augem	1928	—
I. germanica	No fructosan found	(Augem)		
Allium cepa	—	Chevastelon	1894	−25
A. ascalonicum	—	Chevastelon	1894	—
A. sativum	l'Inulin de l'Ail	Chevastelon	1894	−39
A. sativum	Tubéroholoside	†Belval	1939	−39·4
A. scorodoprasium (bulbs)	Scorodose	†Kihara	1935	−41·5 ($\alpha_D^{13°}$)
Asphodelus microcarpus (and other species)	—	*Chevastelon	1894	
A. microcarpus (tubers)	Asphodèloside	Colin & Neyron	1927, 1931	−18
A. microcarpus	Asphodelin	Schlubach & Lendzian	1937	−30·9
Lycoris squamigera	Lycoriside A	Belval	1933	−34
L. squamigera	Lycoriside B or asphodèloside	Belval	1937	−19
L. radiata	—	*Belval	1933	—
Hyacinthus orientalis	—	Colin & Belval	1923*a*	−40
Narcissus pseudonarcissus	Asphodèloside	Belval	1937	−19
Polianthes tuberosa	Tubéroholoside	*Chevastelon	1894	—
		Belval	1939	−38·7
Asparagus (roots)	Asparagosin	Tanret (G.)	1909	−35·1
Asparagus (roots)	Asparagosin	Schlubach & Boë	1937	−32·4
Yucca filamentosa	—	*Meyer	1885	—
Yucca filamentosa	—	Schlubach & Flörsheim	1931	−25·3
Dracaena australis	—	Ekstrand & Johannson	1887	−41·1
Convallaria majalis	—	†*Kylin	1918	—
Agropyron repens (rhizomes)	Triticin	Ludwig & Müller	1872	−49·6
A. repens (rhizomes)	Triticin	Ekstrand & Johannson	1887	—
A. repens (rhizomes)	Triticin	Colin & de Cugnac	1926	−47
A. repens (rhizomes)	Triticin	‡de Cugnac	1931*a*	−48·5
A. repens (rhizomes)	Triticin	Schlubach & Peitzner	1937	−51·4
A. caninum	Triticin	Colin & Belval	1937	−49
—	—	de Cugnac & Belval	1939	−48
Elymus arenarius	Elymoside	†Colin & Belval	1937	−43
Hordeum bulbosum	Hordeoside	Colin and Belval	1937	−43
Phleum pratense	Phlein	Ekstrand & Johannson	1887	−48·1
Phleum pratense	Phlein	de Cugnac	1931*a*	−49
Agrostis alba	Graminin	Ekstrand & Johannson	1887	—
A. alba (stolons)	—	de Cugnac	1931*a*	−45·7
Phalaris arundinacea	—	Ekstrand & Johannson	1887	−48·9
(*Baldingera*)		*de Cugnac	1931*a*	—
Trisetum alpestre	—	Ekstrand & Johannson	1888	−38·9

Table 1 (*continued*)

Plant	Name given to fructosan	Isolated by	Year	Specific rotation α_D^{20}
Festuca	—	Ekstrand & Johannson	1887	—
Calamagrostis	—	Ekstrand & Johannson	1887	—
Arrhenatherum bulbosum	Graminin	Harlay	1901	−44·7
A. bulbosum	Graminin	Colin & de Cugnac	1926	−44
A. bulbosum	—	de Cugnac	1931*b*	−43
A. elatius	Graminin	Issoglio	1921	−38·6
Psamma arenaria	—	de Cugnac	1931*a*	−48
Bromus mollis	—	*de Cugnac	1931	—
Alopecurus agrostis	—	*de Cugnac	1931	—
Lolium italica	—	Schulze	1899	−24·3
Lolium perenne	—	*de Cugnac	1931	—
Rye grass (Western Wolths)	Crude preparation only	Norman	1936	−40
Poa trivialis	Levan?	Challinor *et al.*	1934	−38
Secale (grain)	Synanthrose	‖Müntz	1878	—
Secale (grain)	Lévosine	Tanret	1891	−36
Secale (grain)	—	Tillmans	1928	−43·9
Secale (rye meal)	Graminin	Schlubach & König	1934	−36·6
Secale (rye meal)	—	*Kizel & Keretovisch	1934	—
Secale (stems)	Secalose	Schulze & Frankfort	1895	−28·7
Secale (stems)	Lévosine	Belval	1924	—
Secale (stems)	Lévosine	Colin & Belval	1934	—
Avena (grain, stem & leaf)	—	‖Müntz	1886	—
Avena (grain)	—	Ekstrand & Johannson	1887	−38·9
Avena (grain)	—	Schulze	1899	−31·7
		*Belval	1924	
Triticum (grain, stem and leaf)	—	‖Müntz	1886	—
Triticum (unripe grain)	Lévosine	Tanret	1891	−36
Triticum (stem and grain)	—	*Colin & Belval	1922	—
Triticum (stem and grain)	—	*Belval	1924	—
Triticum (stem and grain)	Lévosine	Colin & Belval	1934, 1937	−36
Hordeum vulgare (grain)	Sinistrin	*Kuhnemann	1875	—
		Kjeldahl	1881	−37
H. vulgare (grain, stem and leaf)		‖Müntz	1886	—
H. vulgare (unripe grain)	Lévosine	Tanret	1891	−43
H. vulgare (stem and ear)	—	*Colin & Belval	1923	—
H. vulgare (stem and ear)	—	*Belval	1924	—
H. vulgare (stem and ear)	—	*Archbold	1938	—
H. vulgare (leaves)	—	Archbold & Barter	1935	−37·8
H. vulgare (leaves)	—	*Yemm	1935	—
H. vulgare (grain and stem)	Lévosine	Colin & Belval	1937	−36

* Fructosan not isolated, presence deduced from estimations of sugars before and after hydrolysis with weak acid and invertase.

† Fructosans are also reported in *Allium vineale* and other species of *Allium* (Belval, 1939; Kihara, 1935*a*, *b*), in *Tulipa* (Belval, 1939), and in *Elymus giganteus*, *E. junceus*, *E. scabulosus*, *E. curvatus* (Colin & Belval, 1937), and in *Tilia europea*, a dicotyledon (Kylin, 1918), and in eight other species of *Scilla* (Colin & Belval, 1923*a*).

‡ According to de Cugnac there are no fructosans in maize, rice or sugar cane, or in the grasses *Molinia caerulea*, *Phragmites communis*, *Arundo Donax*, *Brachypodium pinnatum*, *Cynodon Dactylon*, *Spartina polystachya*.

‖ Müntz' preparations later shown to have been contaminated with sucrose (Colin & Belval, 1923*b*; Belval, 1924).

water, and precipitating the fructosan from the aqueous liquid with alcohol. Numerous modifications in detail have been employed, such as a preliminary treatment with boiling alcohol to inactivate enzymes, the use of clearing agents to remove other water-soluble substances from the aqueous extracts, and the use of baryta to effect purification of the crude product. In recent times acetylation has also been used for the final purification of the product.

Table 2. *Fructosan contents of various plants*

Plant	Part	Period	Fructosan % fresh weight	Authority
Wheat	Leaf sheath	May/June	0·52 – 2·71	Belval (1924)
	Stem	May/July	0·00 – 5·08	Belval (1924)
	Ears	May/July	8·00 – 1·11	Belval (1924)
Rye	Stems	May/July	0·84 – 5·64	Belval (1924)
	Ears	May/July	6·56 – 1·00	Belval (1924)
Oats	Stems	June/July	3·46 – 0·46	Belval (1924)
Barley	Stems	June/July	2·91 – 2·06	Belval (1924)
Elymus arenarius	Stems (base)	Aug./Sept.	11·47	Belval (1938)
E. arenarius	Rhizomes	Aug./Sept.	8·30	Colin &
E. giganteus	Stems (base)	Aug./Sept.	15·02	Belval (1937)
E. scabulosus	Stems (base)	Aug./Sept.	5·64	Belval (1938)
E. curvatus	Stems (base)	Aug./Sept.	1·83	Belval (1938)
Narcissus pseudonarcissus	Bulb	Mar./April	1·14 – 6·00	Belval (1937)
Iris foetidissima	Leaves	Mar./June	3·84 – 8·00	Augem (1928)
I. foetidissima	Rhizome	Dec./June	9·48 – 11·00	Augem (1928)
I. foetidissima	Seed	July	1·80	Augem (1928)
I. pseudacorus	Leaves	—	None	Augem (1928)
I. pseudacorus	Stems	July	2·56	Augem (1928)
I. pseudacorus	Rhizomes	Nov./July	4·56 – 4·68	Augem (1928)
I. pseudacorus	Seeds	June	4·12	Augem (1928)
Lycoris squamigera	Bulbs	Aug./May	3·85 – 11·40	Belval (1933)
L. radiata	Bulbs	Nov./April	4·61 – 11·61	Belval (1933)
Asphodel	Bulb	Feb./June	0·27 – 10·99	Colin & Neyron (1927)

The first detailed account of a reasonably pure preparation is that of Schmiedeberg (1879) who isolated a fructosan from the bulbs of *Scilla maritima* (*Urginea scilla*). The dried and powdered bulbs were extracted with a small amount of water, and normal lead acetate added to the extract to remove proteins, etc. The excess of lead was precipitated with H_2S and the fructosan separated as its calcium compound by the addition of lime. The calcium compound, a pasty mass, was washed with cold water and then treated with carbon dioxide. After warming and filtering off the carbonate, oxalic acid was added to the filtrate to remove the last traces of calcium. The solution was then decolorized with charcoal, concentrated, and the crude fructosan precipitated as a gum by addition of alcohol. The gum was dried over H_2SO_4 and freed from ash by repeated fractional precipitations with alcohol.

The calcium compound is relatively soluble in water, and the addition of lime therefore diminishes the yield considerably. Later procedures, otherwise very similar to Schmiedeberg's, omit this step (e.g. Kjeldahl, 1881; Ekstrand & Johannson, 1888; Harlay, 1901). In some instances the water extracts were so slightly coloured that charcoal clarification was also unnecessary (Harlay, 1901; Augem, 1928; Schlubach *et al.* 1929–37). Harlay (1901) describes this simplified form of

extraction for the isolation of a fructosan from *Arrhenatherum bulbosum* (Avoine à Chapelets). 250 g. of "tubércules" gathered in December were macerated with 300 c.c. of 5% normal lead acetate. After standing for 18 hr. the mass was pressed out, and the expressed liquid allowed to stand for a further 24 hr. The excess lead was removed as oxalate, and after addition of a little $CaCO_3$ the mixture was filtered and 6 vol. of 90% alcohol added. The crude fructosan settled in a solid cake, and was dried over H_2SO_4. The yield was 4·8%.

In a recent preparation of the fructosan from *Agropyron repens*, Schlubach & Peitzner (1937) used 55 kg. of rhizomes. After cutting up the material and grinding it twice in a mortar, it was extracted with 60 l. of water at 50°. The extract was treated with basic lead acetate at 60° and, after removal of the precipitate and of the excess lead, it was concentrated at 30° to 6 l. Alcoholic precipitation yielded 2·9 kg. of crude fructosan. The crude product was reprecipitated many times, until finally no change in the specific rotation was observed on further reprecipitation. The adsorbed alcohol was then removed by evaporating an aqueous solution of the fructosan, which was finally evaporated carefully to a syrup. The final drying consisted in heating the residual syrup in high vacuum at 80° for 2 hr. Acetylation and regeneration of the fructosan did not change the specific rotation.

Since the fructosans are generally extremely soluble in water, they can often be completely extracted with alcohol containing water, the necessary amount of water for a convenient procedure varying from plant to plant. Thus Tanret (1891) employed 70% alcohol for the extraction of immature grains of wheat, barley and rye, subsequently precipitating the crude fructosan by increasing the proportion of alcohol. Tanret found that the barium compounds of cereal fructosans separated readily from fairly dilute solutions, and accordingly purified his products by fractional precipitation with baryta. Belval (1933) used 95% alcohol for the extraction of bulbs of *Lycoris squamigera*, and Schlubach & Koenig (1934) 65% alcohol for rye meal. Schlubach & Flörsheim (1929) extracted dried yucca leaves with methyl alcohol after a preliminary treatment with ether, and claim that all the fructosan was removed. The simple sugars were then removed by fermentation before precipitating the fructosan with alcohol. The barium compounds of the several fructosans vary widely in solubility, some precipitating from aqueous solutions, others only from alcoholic solutions, and a few being soluble even in alcohol. The baryta method has been used by Riche & Rémont (1880), Colin & de Cugnac (1926), Belval (1933, 1939), Colin & Belval (1923*a*, 1937), and Archbold & Barter (1935) for purification of fructosans obtained respectively from *Scilla maritima*, *Arrhenatherum bulbosum*, *Agropyron repens*, *Lycoris squamigera*, *Allium sativum*, *Polianthes tuberosa*, *Hyacinthus orientalis*, *Elymus arenarius* and barley, but Norman (1936) states that this method was unsuccessful with extracts of rye grass, and Belval (1937) reports that both the fructosan of *Narcissus pseudonarcissus*, and "lycoriside B" from *Lycoris squamigera* have soluble barium compounds. Archbold & Barter removed pigments and simple sugars by extracting dried barley leaves with 95% alcohol for a short time before proceeding to the aqueous extraction. Only a small proportion of the fructosan passed into the alcoholic extract.

Colin & de Cugnac (1926) observed that barium is only very slowly removed from its compound with fructosan by carbon dioxide; moreover, precipitation of barium carbonate continues after the solution has become distinctly acid. If, on the other hand, an attempt is made to remove the excess acid with more baryta the neutral liquid after filtration still contains barium. They concluded that $BaCO_3$ is somewhat soluble in solutions of fructosans and recommend the use of H_2SO_4 to remove barium.

Schmiedeberg (1879) found that it was very difficult to remove the last traces of alcohol from his purified product, prolonged drying *in vacuo* over H_2SO_4 being insufficient. He found it necessary to heat the dry substance to 110° to effect removal of the last traces of alcohol. This observation has been confirmed by Schlubach & Loop (1936) who evaporated an aqueous solution of the pure, dry fructosan from *Scilla maritima* several times before all the alcohol could be removed. Subsequently carefully standardized drying conditions were employed to obtain the pure substance from the aqueous solution.

In most instances the yields represent only a small part of the fructosans present, since it is evident that the fractional precipitation methods required to purify a non-crystallizable substance are very wasteful. Thus Müller (1873) obtained only 1–2 % of fructosan from *Agropyron repens* originally containing 6–8 %, and the same yield was obtained by Schulze & Frankfort (1895) and by Tillmans (1928) from rye meal containing about 10 % of fructosan. Schlubach & Loop (1936) only obtained 19 g. of pure substance from 390 g. of the crude product from *Scilla maritima* when fractionation with alcohol was continued until the specific rotation was constant, and Archbold & Barter (1935) obtained 5 g. of pure material after fractionation with baryta of 93 g. of the crude product from barley leaves.

4. PROPERTIES

The general properties of fructosans from the numerous sources quoted in Table 1 are as follows: Fructosans are white amorphous compounds, both odourless and tasteless. They are readily soluble in cold water, forming stable solutions, and insoluble in alcohol. Many are soluble in all proportions in water, but the concentrated solutions may be very viscous, particularly so the fructosans of the Liliaceae. The solubility in aqueous alcohol depends on the proportion of water present. They are non-reducing and non-fermentable, but may be hydrolysed by invertase, different preparations varying considerably in their susceptibility to attack. The rate of hydrolysis is always much lower than that of sucrose under the same conditions. They are, as a rule, resistant to ptyalin and diastase (free from invertase) (but see Kihara, 1935*a*). They are extremely easily hydrolysed by weak acids yielding almost entirely fructose. For phlein, de Cugnac (1931) obtained complete hydrolysis in 5 min. with *N*/700 acid, and for triticin in 10 min. with *N*/10 acid. Fructosans are all *l*-rotatory, the values of the specific rotations of preparations from different sources covering the range $\alpha_D^{20^\circ}$ −19 to −55 (see Table 1).

Fructosans are usually readily precipitated by baryta from fairly dilute aqueous solutions or from 50 % alcohol (Ekstrand & Johannson, 1887; Tanret, 1891; Colin,

1925; Colin & Chaudun, 1933). They may also be precipitated by $Ca(OH)_2$ or $Sr(OH)_2$, but the calcium and strontium derivatives are somewhat more soluble in water (Schmiedeberg, 1879; Schulze, 1899; Colin & de Cugnac, 1926). They are not precipitated by normal lead acetate, and seldom by basic lead acetate, but may be thrown out by the addition of ammonia to the lead acetate. If precipitation does occur with basic lead acetate it is extremely slow, and the precipitate is soluble in excess of the reagent (Archbold & Barter, 1935). They do not colour iodine (Schmiedeberg, 1879; Ekstrand & Johannson, 1888), but HCl vapour gives a purple colour which distinguishes them from polysaccharides not containing fructose units (Colin & de Cugnac, 1926). They are very hygroscopic and take up water on exposure to air, becoming gummy (Colin, 1925; Colin & de Cugnac, 1926).

Fructosans have no defined melting-point, but on heating swell up and decompose with frothing, turning first light brown, then dark, and finally charring. "Melting-points" are given in some of the earlier papers, and the onset of decomposition appears to occur at considerably higher temperatures in some fructosans than in others, owing to the non-crystalline nature of the preparations it has not been considered worth while to quote the "melting-points".

Most fructosans appear to be attacked to a greater or less degree by yeast invertase. It is stated that the preparations from *Arrhenatherum bulbosum*, *Trisetum alpestre*, *Phleum pratense*, together with those from cultivated cereals, and the "lycoriside B" of *Lycoris squamigera*, are fairly readily attacked (Colin & de Cugnac, 1926; de Cugnac, 1931*b*; Belval, 1937; Colin & Belval, 1937), while those from *Agropyron repens*, *Scilla nutans*, *Allium sativum*, *Polianthes tuberosa*, *Elymus arenarius*, *Hordeum bulbosum*, together with the "lycoriside A" of *Lycoris squamigera* are only very slowly attacked (Colin & de Cugnac, 1926; Colin & Chaudun, 1933; Belval, 1933, 1938, 1939). Schlubach & Flörsheim (1929) found that sinistrin B from *Scilla maritima* was resistant to invertase, but Colin & Chaudun (1933) found their preparation readily attacked, and Kihara (1935*a*) finds that fructosans of several species of *Allium* are hydrolysed not only by yeast, but by ptyalin and takadiastase. Archbold & Barter (1935) and Archbold (1938 and unpublished) studied the action of yeast invertase (British Drug Houses preparation) on the fructosans of barley. Using conditions under which sucrose was inverted in an hour (enzyme concentration 0·02%), the degree of hydrolysis in 4 hr. of four fructosan samples varied from 4 to 20%. When the action of invertase on extracts of barley stems and leaves was examined it was found that the hydrolysis curves obtained, representing the production of glucose and fructose by the action of the enzyme, were not consistent with the hypothesis that only sucrose and the fructosan which had been isolated were present. At the outset the rate of production of fructose from sources other than sucrose was greater than that found for any of the fructosan samples; moreover, while the isolated fructosan yielded 95% fructose and 6% aldose on hydrolysis, the ratio of glucose to fructose produced during the first 24 hr. of digestion of the extracts was considerably greater than 6%. In the later stages of hydrolysis the slow production of fructose is consistent with the view that the known fructosan only is being inverted. The course of hydrolysis is an extract of barley

stems, containing about 7 % fructosan, is shown in Fig. 1, with the early part of the curve inset on a larger scale. In this experiment sucrose was completely inverted in an hour, and it will be seen that during this time a considerable excess of fructose over glucose is produced. Glucose production fell almost to zero after 24 hr., but fructose production was not completed for a week. The extract thus appears to contain, in addition to sucrose and the known fructosan, a more readily inverted

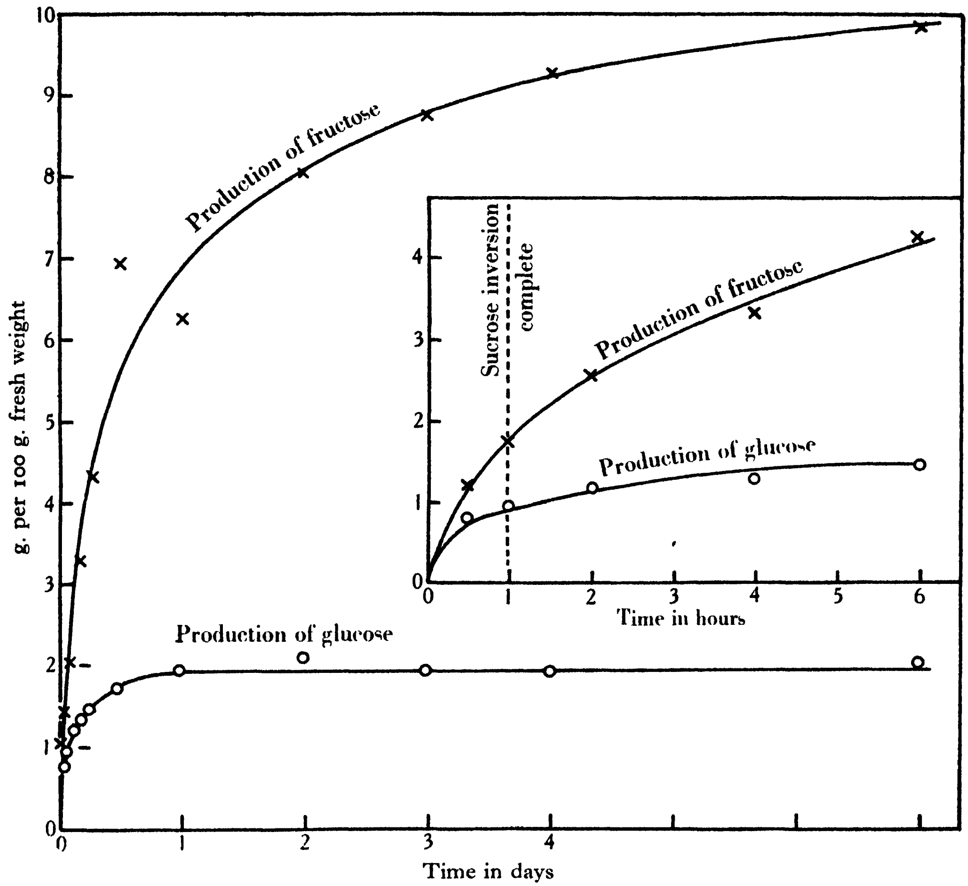

Fig. 1. The production of glucose and fructose by the action of yeast invertase on an extract of barley stems containing 7 % fructosan.

fructosan (or fructosans) which either contains some aldose as an integral part of the molecule, or is accompanied by a polysaccharide yielding aldose. De Cugnac (1931 *a*) made somewhat similar observations for the fructosans of *Arrhenatherum bulbosum* and *Bromus sterilis*.

The view that more than one fructosan may occur in the same plant is supported by the isolation of two substances, sinistrin A and sinistrin B, from *Scilla maritima* by Schlubach & Flörsheim (1929) and Schlubach & Loop (1936), and from *Lycoris squamigera*, lycorisides A and B, by Belval (1933). These products differed only in their solubility in aqueous alcohol, susceptibility to invertase action and in specific

rotation (see Table 1). In cereal plants much still remains to be done before the fructosans are precisely characterized.

It is apparent from the foregoing description that the various preparations exhibit extremely similar properties, differences being observed only in the degree of solubility in water, for example, phlein 3 % and graminin 85 % (de Cugnac, 1931); the temperature at which decomposition begins, susceptibility to invertase action and specific rotation. The degree of similarity between the several fructosans, coupled with the fact that it is exceedingly difficult to prepare a pure sample by repeated precipitation of non-crystalline compounds, early raised the question as to whether the various fructosans were identical or not. Thus Wallach (1888), describing irisin, regards his product as probably identical with the graminin of Ekstrand & Johannson (1887); on the other hand, these authors find that their graminin (from rhizomes of *Trisetum alpestre*) had a solubility several times as great as that of irisin. Many of the earlier samples were doubtless not pure, but even the most careful purification failed wholly to eliminate the differences in properties. Colin, in 1925, summarized the position by stating that monocotyledons contained a number of fructosans of definite types, exemplified by those of *Iris* and *Scilla* on the one hand, which give rather viscous solutions, and those of the grass rhizomes on the other, which are exceedingly soluble in water. The latter group he considers may or may not be identical with the fructosans of cereals. Later, Colin & de Cugnac (1926) attempted to settle the question of the possible identity of the preparations obtained from grass rhizomes by making a careful comparison of triticin (from *Agropyron repens*) and graminin (from *Arrhenatherum bulbosum*), but they did not include a cereal fructosan in their comparison. The results of this comparison are shown in Table 3 and exemplify the differences noted between pure (or practically pure) preparations from different sources.

Table 3. *Comparison of the properties of graminin and triticin (Colin & de Cugnac, 1926)*

	Graminin (*Arrhenatherum bulbosum*, *Trisetum alpestre*)	Triticin (*Agropyron repens*)
Solubility in water	85 %	All proportions
Solubility in 60 % alcohol	12 %	28 %
Strong alcohol	Precipitates rapidly a white mass which sticks to the vessel. (In the presence of 2-3 % ash the precipitate may be powdery)	Precipitates slowly, the liquor are milky at first. A hyaline syrup or a pasty mass separates
Specific rotation	α_D −44 (after hydrolysis α_D −93)	α_D −47 (after hydrolysis α_D −93)
"Melting-point"	Ill-defined. No change to 190°, browns between 190 and 195°, at 199 rises suddenly up tube	Ill-defined. Browns at 160°, at 180° shrinks and begins to decompose, at 198° spreads over tube with frothing
Invertase (yeast)	Readily attacked	Slowly attacked

Both substances are odourless and tasteless, and form calcium and strontium compounds which are fairly soluble in water. They are completely hydrolysed in 10 min. by $N/100$ acid, and yield only fructose. They are unattacked by diastase (free from invertase). They are only precipitated by lead acetate in the presence of ammonia.

These samples were purified by baryta fractionation followed by alcoholic precipitation. Colin & de Cugnac concluded that these two fructosans were really different compounds, and de Cugnac (1931) later extended the comparison to phlein (from *Phleum pratense*) which proved to be different from both graminin and triticin. A further comparison of the properties of the fructosans of wheat, barley and rye with those of *Hordeum bulbosum* and *Elymus arenarius* suggested that these compounds were similar, but probably not identical (Colin & Belval, 1937). From recent work on the constitution, which is discussed in the next section, it would appear that this conclusion that many of the known fructosans differ from one another in constitution is correct. No one fructosan has yet been finally proved to be identical with any other, and it is certain that such substances as scillin, from *Scilla maritima*, irisin from *Iris pseudacorus*, and graminin from rye or grasses all differ in constitution. On the other hand, Haworth *et al.* (1937) have brought forward evidence suggesting that the fructosan of barley leaves, *Poa trivialis* and of *B. mesentericus* may be identical, while Belval (1937) has concluded that the more soluble fructosan of *Lycoris squamigera* (lycoriside B) is probably identical with those of *Asphodelus microcarpus* and of *Narcissus pseudonarcissus*. At present therefore it can only be said that similar types of fructosan occur in plants of similar orders. Apart from rye the cereals have hitherto been rather neglected as a source of fructosan, and no certain conclusion is yet possible either as to the identity of the cereal fructosans one with another, and with those of some grasses, or as to the number of fructosans which may be present in any one plant. Tanret (1891), and later Colin & Belval (1937) appear to have assumed that wheat, oats, barley and rye contain identical fructosans, and that only one is present.

5. CHEMICAL CONSTITUTION

The large proportion of fructose produced by acid hydrolysis of the fructosans, which were initially non-reducing, early led to the view that fructosans consisted mainly of fructose units linked together in such a way that the reducing group of each unit was involved in the linkage (Ludwig & Müller, 1872; Müntz, 1886; etc.). The empirical formula is thus the same as that of cellulose and starch, namely $(C_6H_{10}O_5)_n$. It was then necessary to discover if the molecule consisted entirely of hexose units, and if so whether all the hexose was fructose or if small proportions of glucose (or other sugars) could form an integral part of the molecule.

Colin & de Cugnac (1926) determined the rotation of the hydrolysates of triticin and graminin (see Table 3) and from their results concluded that these substances yielded 100% of hexose. Schlubach & Flörsheim (1929) reached the same conclusion for the sinistrin A from *Scilla maritima* as a result of sugar estimations, while Archbold & Barter (1935) estimated the amount of hexoses produced from the barley leaf fructosan as between 94 and 100%, the exact figure depending upon the number of hexose units in the molecule which was unknown. On the other hand, for the sinistrin B of *Scilla maritima* the value was only 94·4%, for the graminin of rye 91% (Schlubach & Koenig, 1934), and for asparagosin from asparagus roots 92·2% (Schlubach & Boë, 1937). The yield of hexose sugars therefore does not

always reach 100%, although none of the values is much below the maximum. A similar result has been obtained with fructose polymers of the inulin group, and the presence of difructose anhydrides resistant to hydrolysis has been suggested as an explanation (*Ann. Rep. Chem. Soc.* 1930). In the case of the fructosans of the monocotyledons it has been considered justifiable by the workers quoted above to conclude that the molecule does consist entirely of hexose units, which are all liberated by mild hydrolysis. When the values fall short of 100% the low values are accounted for by possible destruction of a little of the fructose during hydrolysis, traces of impurities, and errors of technique in the sugar determinations.

The question as to whether the hexose produced is all fructose has also received a good deal of attention. Tanret (1891), and later Belval (1933) and Colin & Chaudun (1933), found that the specific rotation of hydrolysates of fructosans from cereals, *Lycoris squamigera*, *Scilla maritima* and *S. nutans*, were respectively $\alpha_D^{20^\circ}$ −76, −68, −76, −86, while if only fructose were present the value should be $\alpha_D^{20^\circ}$ −92. Tanret's high values have since been attributed to contamination by sucrose (Belval, 1924), but Belval and Colin & Chaudun believed that in the fructosans of *Lycoris* and *Scilla* there was some aldose sugar in the molecule and later they succeeded in preparing β methyl glucoside from the hydrolysis products of lycoriside A, and the fructosans of *Elymus arenarius* (Belval, 1937; Colin & Belval, 1937). Schlubach *et al.* found only traces of aldose after hydrolysis of several different fructosans, the amounts when added to those of the fructose produced being insufficient to bring the total hexose up to 100%. They considered the molecules contained only fructose, and the low values to be due to destruction during hydrolysis. They do not, however, bring forward any evidence as to whether fructose is actually destroyed under the conditions of hydrolysis. The values they obtained are shown in Table 4.

Table 4. *Hydrolysis products of fructosans (from Schlubach et al.* 1929–37)

Source	Name	Percentage yield of hydrolysis (acid) of		
		Fructose	Aldose	Total
Agropyron repens	Triticin	94·6	0·70	95·30
Rye	Graminin	88·8	0·59	89·4
Scilla maritima	Sinistrin B	94·4	1·8	96·2
Asparagus	Asparagosin	92·2	None	92·2
Asphodel	*Asphodelin	88·9	11·1	100·0

* This substance does not appear to be identical with Asphodeloside (Colin & Neyron, 1927, 1931), see rotation values in Table 1.

Only in the case of asphodel was a considerable proportion of aldose found, but Schlubach inclines to the view that it arose from an accompanying glucose anhydride, rather than from the fructosan molecule which may also be the case with Colin & Belval's products. Finally Challinor *et al.* (1934*b*) found fructose the only product of hydrolysis of the fructosan of *Poa trivialis*, while Archbold & Barter (1935) found 94% fructose and 6% aldose in their purest sample of barley-leaf

fructosan. Unanimity of opinion has thus not been reached on this question, but the trend appears to be in favour of the view that only fructose units are present. Nevertheless, it must be urged that the percentage of aldose in such a case as that of asphodel is rather high to be attributed to impurity, although Schlubach's suggestion of a separate glucose anhydride is a possible explanation. In any case it is clear that, in the main, fructosan molecules consist of linked fructose units.

The advent of Haworth's methylation technique for the study of sugar structure has resulted in a considerable advance in our knowledge of the more detailed structure of the fructosans. In the following account there is given an outline of the results of its application to these compounds; for full details which properly belong to the field of pure chemistry, the reader is referred to the originals of the papers cited below.

The method was applied by Hibbert *et al.* (1931) to the fructosan levan obtained by the action of *B. mesentericus* on sucrose, and later by Challinor *et al.* (1934*b*) and Haworth *et al.* (1937) to the fructosans of *Poa trivialis* and of barley leaves. In these three instances, 1, 3, 4-trimethyl-fructo-furanose was isolated after hydrolysis of the methylated fructosan. These polysaccharides therefore contain fructose in the same form (furanose) in which it occurs in sucrose, and Hibbert & Haworth concluded that they consist principally of fructo-furanose units linked in a straight chain through carbon atoms 2 and 6. The suggested structure is shown below in Fig. 2. It is known that methylinulin yields the 3, 4, 6-trimethyl-fructo-furanose

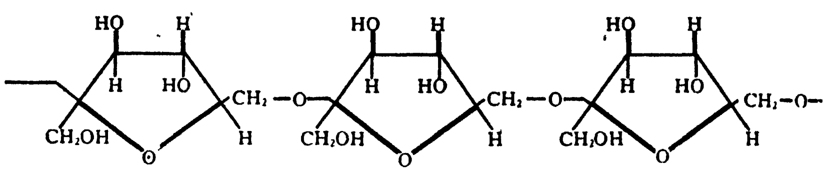

Fig. 2. Linkage of fructo-furanose units in the fructosan of *B. mesentericus* (from Hibbert *et al.* 1931). The linkage of fructose units in the fructosans of *Poa trivialis* and of barley leaves is the same (Haworth *et al.* 1934*a,b*).

on hydrolysis, so that inulin differs from the above fructosans in that the fructose units are linked through carbon atoms 1 and 2, instead of 2 and 6.

Sufficient material was not available for a final decision to be reached as to the number of units of fructose in each molecule or as to the disposition of the end-groups of the chain. Haworth concluded that in all probability these three fructosans are identical and contain about ten hexose units per molecule linked in a straight chain.

Schlubach and his colleagues (1929–37) examined a number of very carefully purified samples of fructosans from various sources not including the three mentioned above. The purification sometimes involved as many as 300 precipitations from alcohol before a product of constant rotation was finally obtained (Schlubach & Peitzner, 1937). The purity of the compound was then checked by acetylation, and comparison of the rotation of the regenerated fructosan with that of the sample before acetylation. With the large amount of material at their disposal they were able to isolate from the hydrolysates of each of their methylated samples not only a

trimethyl fructose, but also the tetramethyl fructose (1, 3, 4, 6) and a dimethyl fructose, and to determine the relative amounts of the three methylated fructoses present. Satisfactory identification of the particular trimethyl and dimethyl fructose isolated was not always achieved, but it is claimed that from the series of fructosans examined two substances of each class were isolated (see Table 5). The isolation of the tetramethyl derivatives of fructose led Schlubach *et al.* to the conclusion that some at least of the fructose units were only linked by one carbon atom, a situation which would occur at the ends of a chain; on the other hand, the isolation of dimethyl fructoses suggests that there must be internal anhydride or ring formation involving a third carbon atom in fructose units already linked to two others. Schlubach suggests that the number of fructose units in each molecule is of the order of ten to twenty and favours a structure which includes the formation of large rings, the degree of ring formation being indicated by the ratio of the tetra-, tri- and dimethyl fructoses found on hydrolysis of the methylated fructosans. A high proportion of tetramethyl fructose in the hydrolysate indicates a structure embodying a central ring with a number of fructose units attached to it by one carbon atom only, a high proportion of trimethyl fructose indicates mainly a straight chain structure and a high proportion of dimethyl fructose a high degree of internal anhydride or ring formation. In Fig. 3 is shown Schlubach's suggestion for the structure of graminin from rye based on the above considerations, and assuming that on hydrolysis of the methylated fructosan the methyl derivatives are produced in the ratio of 2, 1, 2; that the trimethyl fructose is the 3, 4, 6 and the dimethyl fructose the 3, 6.

In three out of the six cases examined the trimethyl fructose isolated is reported to be the 3, 4, 6 derivative that is obtained also from inulin. In the other instances the trimethyl derivative was either methylated in position 1 (and was therefore

Table 5. *Methyl fructoses obtained from some fructosans*

Source of fructosan	Ratio of tetra-, tri- and dimethyl fructoses	Trimethyl fructose	Dimethyl fructose	Suggested number of fructose units per molecule
	(Schlubach *et al.* 1929–37)			
Iris pseudacorus	1 : 0 : 1	II	I	4
Secale (grain)	1 : 1 : 1 (or 2 : 1 : 2)	I (not certain)	I	7–10
Scilla maritima: A	—	I	I	10
Scilla maritima: B	1 : 3 : 1 (or 2 : 5 : 2)	I and possibly II as well	II	15
Agropyron repens	3 : 1 : 3	I	II	Multiple of 7, probably 14 or 21
Asphodel	1 : 5 : 1	Not yet identified	I	At least 7
Asparagus	1 : 8 : 1	I	I	9–10
	(Challinor *et al.* 1934*b*; Haworth *et al.* 1937)			
Poa trivialis (leaf)	—	1, 3, 4	—	About 10
Hordeum vulgare	—	1, 3, 4	—	About 10

All of Schlubach's fructosans yielded 1, 3, 4, 6-tetramethyl fructose.
Trimethyl fructose I is 3, 4, 6 and is also obtained from inulin.
Trimethyl fructose II has position 1 methylated and is probably 1, 3, 4.
Dimethyl fructose I has position 6 methylated and either 3 or 4.
Dimethyl fructose II has position 1 methylated, other methylated carbon not known.

probably the 1, 3, 4; cf. Challinor *et al.* (1934*a*,*b*)), or reported as not certainly identified. Of the two dimethyl fructoses one was methylated in position 1, and the other in position 6 and either 3 or 4. These results are summarized in Table 5, which gives the ratios of the methyl fructoses obtained from various sources and their characterization as far as known.

The possible presence in some methylated fructosans of 3, 4, 6-trimethyl fructose residues suggests that the clear-cut distinction in structure between the fructosans examined by Haworth and inulin may not hold for the whole range of

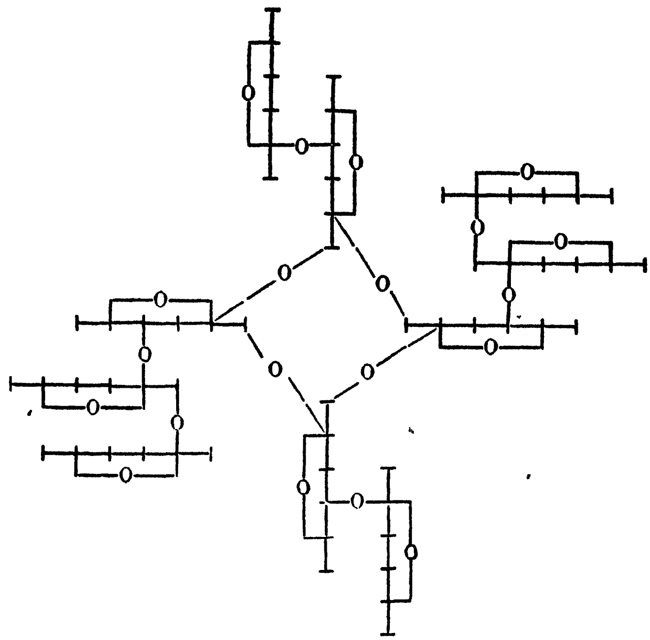

Fig. 3. A suggested formula for the graminin of rye (from Schlubach & Peitzner, 1936). The formula is based on a molecule of ten hexose residues. It is in accord with the observation that the methylated fructosan yielded tetramethyl, trimethyl, and dimethyl fructoses in the ratio 2 : 1 : 2 on hydrolysis. The above compound would yield 3, 4, 6-trimethyl fructose and 3, 6-dimethyl fructose.

fructosans found in the monocotyledons. At present the results of the German workers have not been confirmed elsewhere, and it is admitted that some of the identifications of the methyl fructoses are not conclusive (e.g. Schlubach & Koenig, 1934). If Schlubach's claim to have isolated the 3, 4, 6-trimethyl fructose from the hydrolysates of methylated fructosans is substantiated, the structural difference of these fructosans from inulin will lie only in the number and arrangement of fructose units linked by the same carbon atoms (1 and 2), as compared with units linked by different carbon atoms (2, 6) in the samples examined by Haworth.

6. QUANTITATIVE ESTIMATION IN PLANT TISSUES

Quantitative determinations of fructosans with the object of studying their seasonal changes have been carried out by Colin and his collaborators in France and by Archbold, Barnell and Russell in this country. In the absence of detailed

knowledge of the number of fructosans which may occur in any one plant the quantitative estimation of these substances as a group resolves itself into the estimation of the fructose obtained by hydrolysis with weak acid (not stronger than $N/5$). The methods at present available are all subject to some degree of error owing to the difficulty of separating the products of hydrolysis of the fructosans from those of sucrose.

Although fructosans are relatively insoluble in aqueous alcohol, as compared with sucrose, fructose and glucose, it is not possible to effect complete extraction of these sugars without removing some fructosan. The simpler sugars and the fructosans must therefore be obtained in one extract and then separately estimated (Augem, 1928; Archbold, 1938). The possibility of enzyme action naturally precludes the use of cold water on fresh material, but Norman (1936) has used cold water for the extraction of dried rye grass. Such a procedure may be unsatisfactory, since the rapid drying conditions usually found necessary to avoid hydrolytic changes may only partially destroy the plant invertase (Archbold, 1938). Immediate treatment of the fresh material with strong alcohol (95 %) is therefore to be recommended (Archbold, 1938; de Cugnac, 1931; Belval, 1924). After boiling the fresh material for 10 min. in alcohol, the alcohol may be diluted with water to 60 or 70 % and an exhaustive extraction completed. Alternatively, the alcohol may be poured off and the tissue then extracted with cold water. The alcoholic fraction is, in the meantime, freed from alcohol and the residual liquid added to the water extract. Colin & Belval (1922) and Belval (1924, 1933) used exhaustive alcohol extraction to remove sugars (including fructosans) from wheat, barley and rye plants and from *Lycoris* bulbs, and state that complete removal of fructosans was obtained (but see de Cugnac, 1931*a*). Augem (1928) used water extraction following a preliminary treatment with alcohol in an unsuccessful attempt to separate fructosans from other sugars in iris rhizomes, and finally adopted 70 % alcohol as the extracting agent. Barnell (1938) treated the residue left after alcoholic (80 %) extraction of wheat plants with water (temperature not stated) for 48 hr., and then estimated fructosans in the aqueous extract. There is little doubt that some fructosan will pass into the preliminary alcoholic extract when fresh material is treated in this way, since precipitation does not occur readily when the relatively dilute solution in the plant is further diluted with alcohol. In Barnell's experiments therefore the sucrose estimate, made after hydrolysis by $N/2$ HCl at 60° C., will include the fructosan in the alcoholic extract, and the estimates of fructosan in the subsequent aqueous extract will accordingly be too low. Archbold (1938) and Russell (1938) used cold water following alcohol for the extraction of sugars from barley plants. When a micro-method of sugar determination was used 10–20 g. of fresh material were first boiled with about 150 c.c. of 95 % alcohol and subsequently extracted with 150–300 c.c. of water and made up to final volumes of 250–500 c.c. Whichever technique is adopted for extraction an aqueous solution of all the sugars, together with other plant products soluble in water and alcohol, is obtained. The solution is always coloured (except for grain extracts) and is frequently opaque.

The necessity of obtaining clear solutions for polarimetric readings led to the

use by the French workers of normal lead acetate as a clarifying agent. For the estimation of sugars in extracts of leaves and stems of barley where sugar determinations did not involve the use of the polarimeter, Archbold (1938) recommends unclarified solutions, and a suitable micro-copper reagent. Experiments with various clarifying agents are discussed in detail, and also the errors involved if clarification is omitted.

After determination of free glucose and fructose separate estimates of fructosan and sucrose can be attempted in two ways. The first utilizes the selective action of invertase on sucrose, fructosan being determined as the difference between the sugar produced by acid hydrolysis and by invertase hydrolysis. It has already been pointed out (p. 193) that many fructosans are somewhat susceptible to invertase, and it is clear from Fig. 1 that where a high proportion of fructosan is present the errors may be large if it is assumed that the reducing sugar resulting from invertase action arises solely from sucrose. Sucrose will be considerably over-estimated, while fructosans will be under-estimated, the percentage errors on each fraction depending on the relative amounts present.

In their quantitative analysis the French workers have used this method exclusively. In justification of its use Augem (1928) makes the general statement that fructosans are on the whole resistant to invertase action, while Colin & Belval (1922), and Colin (1925) mention particularly that if extracts of bluebell leaves or wheat plants are subjected to invertase action part of the sugar remains uninverted. The resistant portion is identified with the fructosan, and no indication is given that the resistance to inversion is only a relative one. Later, however, they do report that the fructosans of *Scilla* (Colin & Chaudun, 1933), of *Lycoris* (Belval, 1933) and of some grasses (de Cugnac, 1931*a*) are more or less readily inverted by invertase.

The measurements made on the clarified extracts may be summarized as follows (Belval, 1924). (1) Rotation (α_1) and copper reduction of the solution before hydrolysis. (2) Rotation (α_2) and copper reduction of the solution after hydrolysis with invertase. (3) Rotation (α_3) and copper reduction of the solution after hydrolysis with dilute acid. The difference $\alpha_1 - \alpha_2$ and the difference between the copper reducing powers (2-1) give two independent measures of sucrose, and $\alpha_2 - \alpha_3$ and the difference between the copper reducing powers (3-2) give two measures of fructosan, on the assumption that only excess fructose is produced by the acid hydrolysis.

Belval claims that the values obtained for sucrose by the two methods agree sufficiently well to justify the conclusion that the hydrolysis of fructosan by invertase is negligible under the conditions he employed. Archbold (1938) found errors up to 30 % in estimates of the sucrose in barley plants containing much fructosan when invertase (0·02 %) was used. Even if allowance is made for differences in activity of the several enzyme preparations, it seems likely that Belval's values for fructosans (some of which are quoted in the sequel) are too low. It may perhaps be pointed out that both his methods of determining sucrose will lead to over-estimation in the presence of fructosan, since change in rotation due to fructosan hydrolysis will increase the negative rotation of the hydrolysate more than an

equivalent amount of sucrose, a fact which may account in part for the agreement between the sucrose estimates found by Belval.

The second possibility for estimating fructosan is the separate determination of the increases in glucose and fructose after acid hydrolysis. Its success depends on the absence of sources of glucose, other than sucrose, which will yield glucose under the conditions appropriate for fructosan hydrolysis. In these circumstances sucrose may be determined as twice the glucose increase, and fructosan as the difference between the increase of fructose and of glucose. This procedure was used by Archbold (1938) and Russell (1938) in their work on barley, and for many purposes gives satisfactory results. An unsatisfactory feature became evident in the analysis of stem samples collected during the later stages of growth when it was found that appreciable amounts of glucose were produced which could not have arisen from sucrose (see Fig. 1). In some of these cases the estimate of sucrose even exceeded that obtained as a result of invertase hydrolysis. In a general comparison of the two methods it was found that estimates of sucrose and fructosan were in agreement when the amount of fructosan present was small (as in many leaf samples), in the majority of instances lower estimates of sucrose, and consequently higher estimates of fructosan were obtained when the acid hydrolysis method was used as compared with invertase hydrolysis, but in the stem extracts mentioned above the position was reversed. More information as to the sources of glucose, at present undefined, is required before a completely satisfactory technique can be devised. In the barley it appears unlikely that raffinose is present, and the presence of a fructosan containing some glucose in the molecule has been suggested as a possibility.

Norman (1936) also used the separate determination of glucose and fructose for the estimations of fructosans in rye grass. His procedure was to oxidize the glucose and other susceptible substances with hypoiodite before and after acid hydrolysis, and in each case to determine the residual fructose by means of a copper reagent. The difference between the two fructose estimates was termed fructosan. It is clear that since fructose produced from sucrose will be included, this estimate of fructosan can only be an approximate one. Judging from the small increase in aldose on hydrolysis, as determined by the hypoiodite values, Norman concluded that little sucrose was present. In view of the fact that young leaves generally contain large amounts of sucrose relative to other sugars this conclusion appears to require further proof although the explanation may be that sucrose was largely hydrolysed during the water extraction of the dry tissue (see p. 201). The hypoiodite method of determining glucose is not a very sensitive one, and in addition the "apparent glucose" values obtained in uncleared extracts are largely due to substances other than sugar. The aldose increase is therefore a small difference between two large values, and no account is taken of any possible effect of the acid hydrolysis on substances other than aldose which are oxidized by iodine.

At the present time the colorimetric methods available for fructose estimation have not been applied to the problem of fructosan estimation, largely owing to the difficulties of removing other sources of colour without loss of fructose.

7. Seasonal changes in fructosans

The probable importance of fructosans in carbohydrate metabolism was fully realized by Colin, who initiated a series of investigations into their distribution first in cereals (Colin & Belval, 1922; Belval, 1924), later in grasses (de Cugnac, 1931 *a, b*) and in such plants as *Lycoris* (Belval, 1933), *Asphodelus* (Colin & Neyron, 1927) and *Iris* (Colin & Augem, 1927; Augem, 1928). These workers stress the point that owing to the solubility of the fructosans in water, the seasonal fluctuations in amount should be considered principally in relationship to those of the simple sugars, and further that they should be regarded as storage material only in the same transitory sense as, for example, sucrose is so regarded in the sugar cane. The following account is largely taken from the work of Colin and his collaborators and from that of Archbold (1938).

(*a*) *Seasonal changes in fructosans of cereals and grasses*

Colin & Belval (1922) and Belval (1924) analysed leaves, stems and ears of wheat, barley, rye and oats at various stages during growth. In some experiments leaf sheaths and leaf laminae were analysed separately, and the rachis of the ear was also separated from the grains. In the leaf laminae only sucrose and its products of inversion were found, and the proportion of sucrose was very high (70–80 % of the total sugar). Fructosans appeared in the leaf sheaths, the amount rising, in wheat, from 0·52 % (fresh weight) on 19 May to 2·56 % on 11 June, the latter value representing 50 % of the total sugar present.

In the stems before the spikes appeared fructosans were again absent, but the ratio of sucrose to hexose was lower than in the leaves. As the spikes emerged fructosans began to accumulate in the stems and reached a maximum concentration at the flowering period. In wheat stems (1922) the value reached was 5·08 %. When the leaves began to die off, sugar concentration and with it fructosan concentration fell, until at harvest only little sugar remained. During this latter phase the relative amount of hexose rose slightly. Analyses of upper and lower halves of the stems during sugar accumulation showed that fructosan concentration tended to be greater at the base of the stem than at the apex, while that of hexose was greater at the apex. The rachis contained fructosans in lower concentration than the stems, and in less amount relative to hexoses. The maximum amount was 25 % of the total sugar as compared with 50 % in the stems.

Ears were separated as soon as they were big enough to be excised readily, and had at the outset a high concentration of fructosan (6–8 % of the fresh weight). The concentration fell continuously throughout ear development. Two sets of Belval's data, one for wheat, one for rye stems and ears, are shown graphically in Fig. 4.

Qualitative confirmation of Colin and Belval's results has been obtained by Barnell (1938) who reports the presence of fructosans not only in the stems, ears and leaf sheaths of wheat (rivets) but also in the leaf laminae. He found an accumulation of fructosan in the stems during growth, the amount reaching a maximum in early July. Concentration was higher in the lower part of the stem than in the

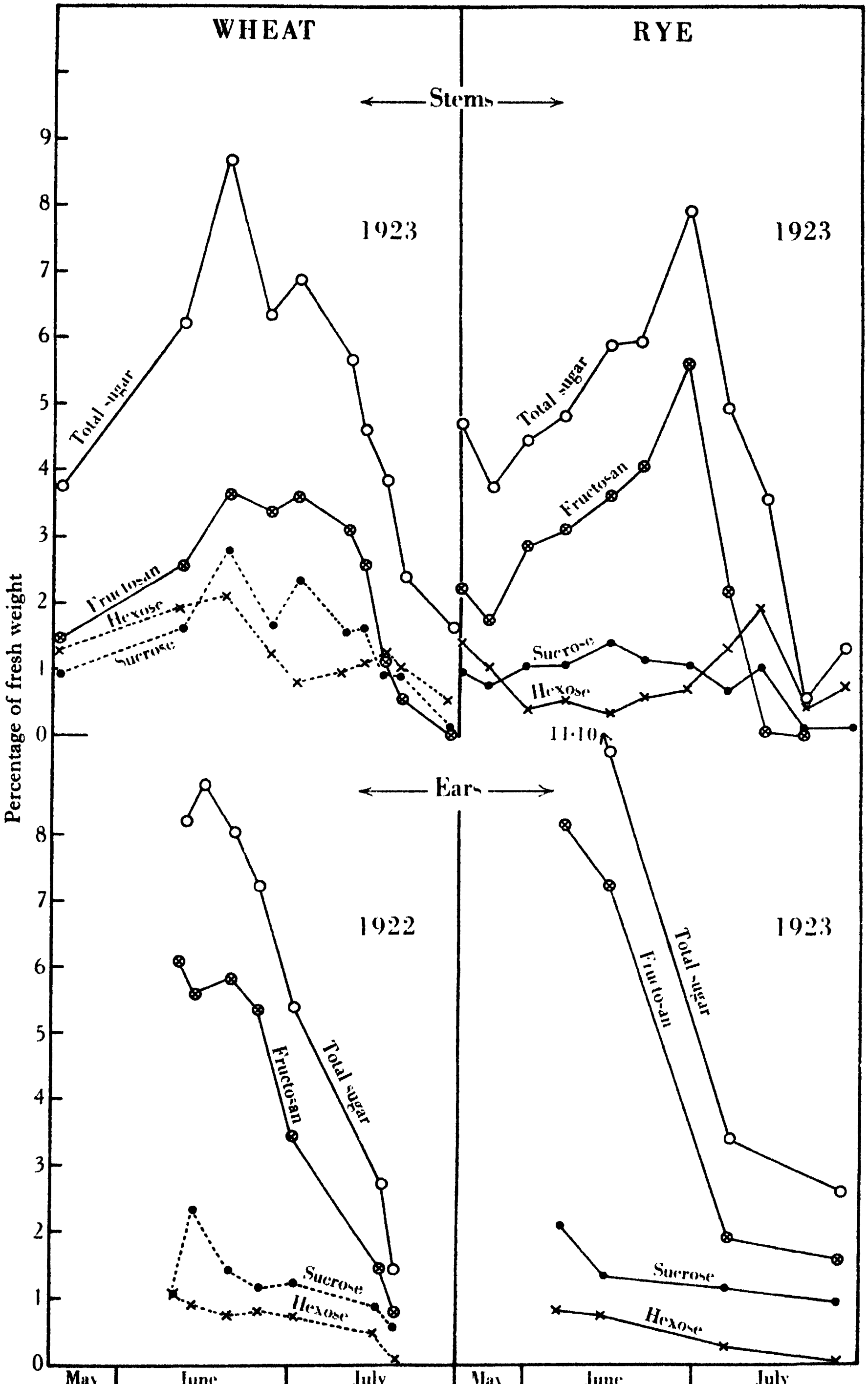

Fig. 4. Concentration changes in fructosans and other sugars in the stems and ears of wheat (Blé des Alliés) and rye (Géant de Russie) during growth. Plants harvested at the end of July (plotted from the data of Belval (1924)).

upper in June, but in July the reverse was the case. In the ears the initial concentration was high, but fell continuously during growth, while in the leaf sheaths the concentration rose to a maximum in early June and then fell. In the leaves there were no important fluctuations in fructosan concentration. Although the seasonal drifts in fructosan content described by Barnell are doubtless correct, no reliance can be placed on the actual values recorded, which are in fact much lower than those of Belval, since the technique adopted must result in the inclusion of a fair proportion of the fructosan present in the sucrose estimate, with consequent low values for the fructosan (see p. 201).

Similar experiments were carried out on barley by Archbold (1938), which on the whole confirmed the results discussed above, and also included a study of sugar changes in the separate internodes of the stem. By the isolation of a fructosan from a large sample of barley leaves (Archbold & Barter, 1935), special attention was focused on the question of the occurrence in leaves. It was found that fructosans were usually present, though often in only small amounts. This observation constitutes the only important difference from the results of Colin and Belval. At the time of emergence of the third leaf the fructosan in the leaves was slightly in excess of hexoses and constituted about 30% of the total sugar. The concentration rose slowly until rapid stem elongation began when it fell almost to zero. Subsequently there was a secondary rise, partly due to water loss from the leaves which becomes rather rapid at the stage of ear development. The concentration at this late stage was 0·7% of the fresh weight, the total sugar being 2·5%. It will be shown in the next section that the amounts of fructosan are much affected by environmental conditions, and may be much higher than that quoted above.

In the barley stem fructosan was present in the early stages, as well as during the development of the ear (cf. wheat, Belval, 1924). At first the amount was equal to that of the hexoses and constituted about 40% of the total sugar, later there was an accumulation of fructosan (and total sugar) followed by a fall similar to that described by Belval. The maximum concentration was 4% of the fresh weight and the proportion of the total sugar about 50%, figures very similar to those for wheat.

From the analyses of the separate internodes of the stem it was found that, as the last two internodes elongated, fructosan began to accumulate rapidly in those below, and a gradient of increasing fructosan concentration was established from apex to base, the gradient of sucrose concentration was in the same direction, while that of hexoses increased from base to apex. When stem elongation was complete fructosan storage was greatest in the large upper internodes and the maximum concentration shifted to the middle of the stem (see Fig. 5; cf. Barnell's results for wheat, p. 205). At the same time the amount of fructosan relative to other sugars increased markedly, indicating that at this stage most of the excess sugar arriving from the leaves was being stored in this form.

No comparable data for roots are available, but a few analyses of roots have been made by Russell (1937). Samples were collected at the third, sixth and ninth leaf stages, from plants grown in sand culture under various conditions of manurial deficiency. In many of the early samples the amount of glucose liberated on hydro-

lysis exceeded that of fructose, so that the presence of fructosans could not be demonstrated. At the later stages of growth, however, there is an excess of combined fructose over glucose, and it is thus established that fructosans can exist in the roots.

De Cugnac (1931 *a*) examined 38 species of Gramineae at the time of flowering and found fructosans in 23 (see Table 1). They were present in all the aerial parts except the leaf laminae (but see p. 202), and values ranging from 1 to 17% of the fresh weight are recorded for the stems. The seasonal history of *Arrhenatherum bulbosum*, *Agrostis alba* and *Agropyron repens* was similar to that of cereals, namely, an increase of fructosan in the stems, particularly at the base, until flowering, followed by a fall, presumed to be due to migration to the ear. The ears had a high concentration in the early stages of development, while in the perennial parts there was always a considerable amount which persisted throughout the year (see also p. 212).

As a result of all their investigations the French workers conclude that cereals and grasses can be divided into two groups, those which form fructosans and those which do not. Among the cereals, wheat, barley, rye and oats belong to the former category and maize and rice to the latter. Since no fructosans were found in the leaf laminae of cereals it was concluded that formation of these substances began in the leaf sheath and therefore they must be secondary products of sugar change and not primary assimilates. With this view Archbold agrees, for although fructosans were found in barley leaves additional evidence (see p. 215) was obtained from a study of the effect of environmental factors on fructosan content indicating that it was of secondary origin. Nevertheless, exception must be taken to the repeated and emphatic statements of Colin *et al.* that fructosans never appear in the leaves of cereals (Colin & Belval, 1922; Belval, 1924; Colin, 1925). It is possible that their analytical technique which overestimates sucrose at the expense of fructosan may have failed to detect the sometimes rather small amounts in leaves. Alternatively, the cultural conditions under which the plants were grown may have been such that the general concentration of soluble carbohydrates was low and fructosans were not formed in the leaves, a situation found in barley by Yemm (1935) in a "poor carbohydrate" year, and by Archbold (1938) when large doses of nitrogen were given.

In the stems of both wheat and barley the ratio of sucrose to other sugars is much lower than in the leaves where sucrose is throughout the predominant sugar. Assuming that sucrose is exported from the leaf it must presumably be converted directly or indirectly into other sugars during transport or at the seat of temporary storage in the stem. If inversion were the only change which took place the concentration of hexoses should increase towards the base, while actually the reverse is the case. It appears, therefore, that hexoses are converted to fructosan, and that this sugar and unchanged sucrose accumulate. Additional support is given to this view by Belval's observation that in maize which does not form fructosans, hexoses increase in the sheaths, while in wheat he found fructosans in the leaf sheaths.

The stage at which the leaves begin rapidly to die off coincides with the most active development of grain, and a large demand for carbohydrates. The stored

sugars in the stem are therefore drawn upon and sugar concentration falls. In the experiment depicted in Fig. 4 three-quarters of the maximum amount was used up by harvest time. The relative increase in hexoses during this stage of sugar depletion led Belval to conclude that fructosans themselves are not translocated but undergo hydrolysis and migrate as hexoses. He points out, however, that the expected lowering of the rotatory power of the stem extracts which should follow the liberation of free fructose does not occur, and suggests as an explanation either conversion of fructose to glucose or preferential utilization of some of the fructose in polysaccharide synthesis. An alternative explanation would of course be preferential translocation of fructosans. The barley experiments were not continued sufficiently long to obtain certain evidence of fructosan migration from the stem, but there was evidence that it could be translocated from the ear sheath, and it is doubtful if hydrolysis is essential before sugar migration. Since fructosans are found quite abundantly in the ears, it would be simpler, in the absence of proof to the contrary, to adopt the hypothesis that fructosans are translocated as such from the stems to the developing ears and are there re-utilized, a point of view which is implicit in de Cugnac's (1931 *b*) description of the fructosan metabolism in grasses. In any case, it is clear that fructosans are stored only temporarily in the stems during a relatively short period when the leaves deliver more sugar than is immediately required for ear growth.

In the ears the rapid fall in fructosan concentration is said to indicate that the starch reserves are built up at the expense of fructosan (Belval, 1924). Such a conclusion cannot be justified on the basis of concentration data only, the falling concentration may be only an expression of the fact that fructosans do not increase in proportion to the total weight. In any case, in Belval's wheat samples (Blé des Alliés, 1922), while fructosan concentration fell from 6·07 to 0·76%, starch concentration rose from 4·94 to 61·84%, so that even if the problem of growth of the ear were not involved, only a small fraction of the starch could be formed from the fructosan found in the ear at the first collection. In barley (Archbold, 1938), where it was possible to calculate the amount of fructosan present in terms of the weight per ear, the absolute amount was found to increase at least until the maximum ear weight was reached, while concentration fell as in wheat and rye. No certain evidence is therefore yet available as to the fate of the fructosan either found in the ear, or translocated to it from the stems. At present all that can be said is that fructosans may be in part the precursors of starch.

The seasonal changes in concentration, taken as a whole, indicate that under normal conditions of growth there is an increase of fructosans in the whole plant at least until stem elongation is complete. Subsequently the amount falls as the total sugar declines, and the ear ripens, but there is no conclusive evidence as to whether the fructosans are re-utilized in respiration, polysaccharide or even protein synthesis. The leaves contain only a small fraction of the total, the bulk accumulating in the stems and ears, where they probably arise from hexoses, produced by inversion of translocated sucrose. They are thus secondary products of sugar change.

When the results are expressed on a per plant basis, it is possible to extend the picture of sugar changes arrived at from the consideration of concentration data. Thus it becomes evident that the secondary rise in fructosan concentration in barley leaves is due to water loss and that the absolute amount of fructosans does not change during the 2 or 3 weeks before the leaves die off, but ultimately the amount begins to fall.

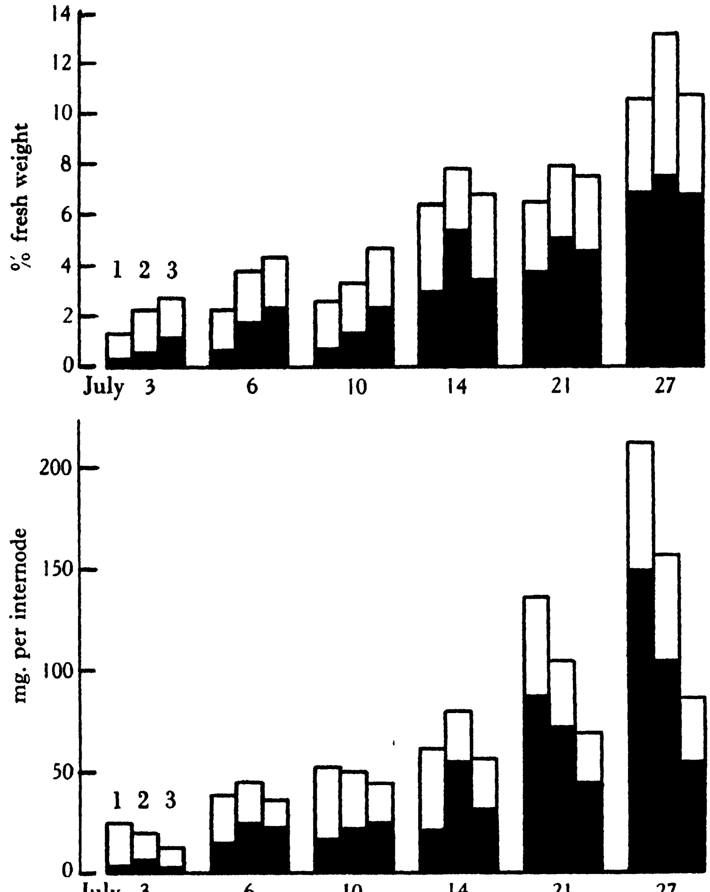

Fig. 5. Fructosans and total sugars in the stem internodes of barley (Plumage Archer) during ear emergence. 1 and 2 are the two top internodes, 3 is the average of the third and fourth internodes from the top (from data of Archbold (1938)).

Total sugar, unshaded; fructosan, black.

In the stems the accumulation of fructosans, followed by a fall, is again emphasized, and in addition it is found that as the large upper internodes reach their maximum size, storage of sugars in them exceeds that in the lower internodes which, nevertheless, have the highest concentration. As the growth of each internode ceases accumulation of hexoses and sucrose in it also ceases, and all the in-

coming sugar is stored as fructosan, so that finally the proportion of fructosan may reach 70% of the total sugar. When stem growth is complete the relative amounts of sucrose and hexoses are highest in the uppermost internodes which continue the longest to receive sugar direct from the attached leaves. The fructosan is thus presumably formed from temporarily immobilized sugar at a relatively slow rate. The concentration and absolute amounts of fructosan and total sugar in the stem internodes of barley (1936) during ear emergence are shown in Fig. 5. It will be seen that while concentration rises from apex to base, the absolute amounts in the internodes are at first nearly equal, but gradually the increase becomes greater in the upper internodes than in the lower. By the time the maximum sugar value is reached the bulk of the fructosan is stored in the upper part of the stem.

In the ear sheath before the awns emerge 50% of the total sugar is fructosan, but as ear growth proceeds the fructosan disappears rather rapidly, while there is little change in sucrose and reducing sugar. The amount of fructosan lost is much larger than could reasonably be assumed to result from respiration losses, and it must therefore be translocated away, presumably to the ear. It is at present premature to discuss in more detail the origin of the fructosans in cereals, since hypotheses based on the preferential use of glucose or fructose for synthesis or respiration cannot be substantiated unless the whole sugar change throughout the plant is known. It may, however, be pointed out that the maximum accumulation of fructosan takes place in any stem internode after its growth has ceased, so that it seems that at this stage all the sugar delivered to an internode is converted to fructosan, involving conversion of glucose to fructose. If preferential utilization of glucose for synthesis occurred then the accumulation of fructosan should be greatest during elongation. The utilization of all the sugar delivered to the stem for synthesis, followed by a slow change of both glucose and fructose to fructosan when stem growth ceases, is at present the hypothesis best fitting the facts.

(b) *Seasonal changes in the fructosans of bulbs and rhizomes*

Sugar changes in rhizomes of *Iris*, *Lycoris* and *Asphodelus* have been studied by Colin & Augem (1927), Augem (1928), Belval (1933) and Colin & Neyron (1927, 1931). In the iris it is found that fructosans may exist together with starch in the rhizome as in *Iris foetidissima*, or as the sole reserve of hexose polymers as in *I. pseudacorus*, or starch alone may be present as in *I. germanica*, while in *Lycoris squamigera* much starch is present together with the fructosan. The seasonal changes of sugars in the rhizomes of iris and lycoris are shown graphically in Fig. 6, and of *Asphodelus microcarpus* in Table 6.

In *Iris pseudacorus* the rhizomes contained about 4% (fresh weight) of fructosan and small amounts (less than 0·5%) of hexoses and sucrose from July to December, when there was a slow fall to about 3% by the following May. In *I. foetidissima* (rhizome de l'année) the percentage of reserve carbohydrate was far higher, and from November to June both starch and fructosan concentrations rose continuously, at the last analysis being 14·5 and 9% respectively. In *Lycoris squamigera* starch and

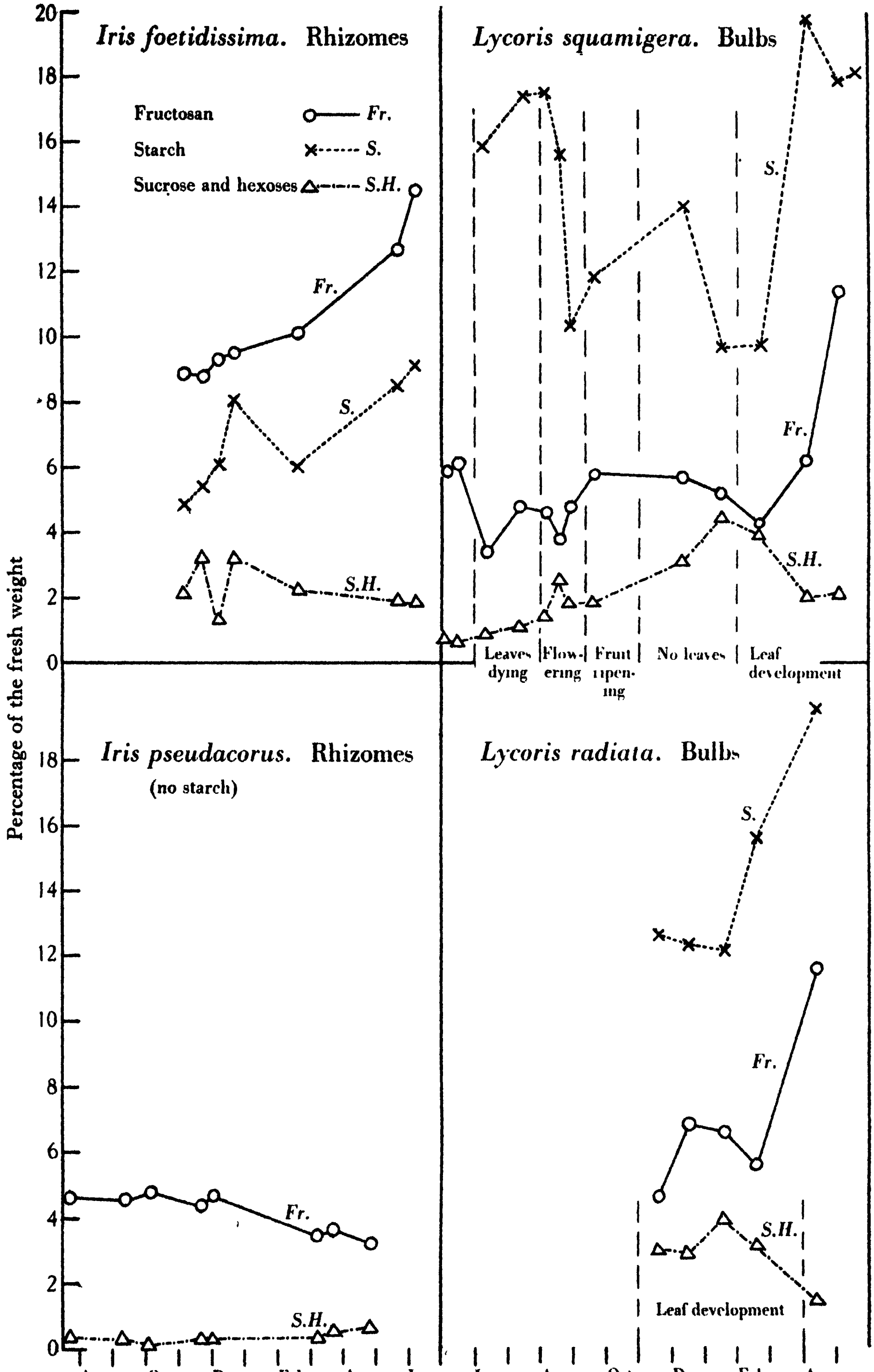

Fig. 6. Fructosans, starch and other sugars in the rhizomes of *Iris pseudacorus*, *I. foetidissima*, and *Lycoris radiata* (plotted from the data of Augem (1928) and Belval (1933)).

Table 6. *Seasonal variation in total sugar and fructosan in the "tubercules" of asphodels (percentage of the fresh weight) (from Colin & Neyron, 1927)*

	Total sugar	Fructosan		Total sugar	Fructosan
February	2·76	0·27	November	8·38	7·88
April	5·28	2·83	December	5·84	3·84
May	10·90	9·85	January	4·00	2·00
June	12·80	10·99	February	2·50	1·10
September	8·30	7·82	April	6·70	3·68

fructosan concentrations rose during leaf development (February to May), fell again as the leaves died off and the flowers appeared (June to August), and remained at the lower value, about 4 %, during development and ripening of the fruits (August to September) and until leaf growth began again in February. The maximum values were reached in May and were starch 19 % and fructosan 12 %. In the asphodel high values of fructosan are found after the cessation of vegetative activity in the summer. In the late autumn the amount falls and also the proportion in the total sugar; a minimum value of about 1 % is reached in February.

In addition to the fructosan in the rhizome, Colin & Augem also found a little at the base of the leaves of iris, and in the inflorescence axis while the seeds are ripening, as well as in the seeds themselves. They give the following values for *Iris foetidissima*: leaves in March 3·84 %, in June 8·00 % and seeds in July 1·80 %. In *Lycoris radiata* Belval found fructosans throughout the leaves, but always in higher concentrations at the base than at the apex, while in *L. squamigera* they were only found at the bases of the older leaves. He attributes this difference to the fact that the leaves remain much longer on the plant in the former case, and are thus able to accumulate a reserve of carbohydrate. In both cases the amounts tended to increase with age, but were usually only of the order of 1 %, sucrose, as is usual, being the predominant leaf sugar.

During the formation of new shoots the above authors find little change in the carbohydrates of the rhizomes, but at flowering there is some loss of the reserves, and Augem makes the interesting observation that it is only at this stage that an enzyme is present in the rhizome which can hydrolyse fructosan, although the leaves always possess this capacity. The conclusion reached is that, as in the cereals, fructosans are formed from hexoses delivered to the rhizomes, and are found also in the base of the leaves, since there is no clear distinction between the leaf base and rhizome. They accumulate during the vegetative development of the leaves, and are used up, in part, for seed development. When new leaves again develop storage of fructosan starts anew. From the data of Fig. 6 it seems that the reserve carbohydrate is far in excess of the subsequent requirements for flower and seed development and much therefore remains unused. It will be remembered that in the rhizomes of *Agropyron repens* a similar situation was found.

8. Effect of manurial deficiency on fructosan content of barley

A rather low fructosan content in barley plants which had received an excessive dose of nitrate indicated that when total sugars were low (as in plants receiving much nitrogen) the fructosan content would also be low. Following on this observation experiments were carried out to test the effect of nitrogen deficiency on fructosan content (Archbold, 1938), while the study of the effects of potassium and phosphorus deficiencies were included (Russell, 1937, 1938) in a general investigation into problems of manurial deficiency already in progress.

Table 7. *Total sugar and fructosan contents of barley (var. Plumage Archer) leaves. Stems and ears from plants grown in sand and receiving nitrogen-deficient dressings (from Archbold,* 1938)

Results are averages of duplicate analyses carried out on three occasions, 8 July 1937, 15 July 1937 and 26 July 1937.

	Leaves (% fresh wt.)		Stems (% fresh wt.)		Ears (% fresh wt.)	
	Control	- N	Control	- N	Control	- N
Total sugar	1·52	3·04	2·03	5·76	4·79	4·81
Fructosan	0·13	1·15	0·43	4·03	2·22	2·47

Table 8. *Mean effect of potassium and phosphorus deficiency and of the sodium/calcium balance on fructosan concentration in barley (var. Plumage Archer) leaves and stems (from Russell,* 1938)

Results are averages of duplicate analyses carried out on three occasions, 5–8 June 1936, 19–24 June 1936 and 9 July 1936.

Fructosan as percentage of fresh weight

Potassium			Phosphorus			Sodium/calcium balance		
	Leaves	Stems		Leaves	Stems		Leaves	Stems
High	0·93	2·02	High	0·28	0·86	High Na	0·45	1·01
Medium	0·53	1·19	Low	1·82	1·44	High Ca	0·65	1·29
Low	0·18	0·24						

The effect of nitrogen deficiency on fructosan and on total sugar contents of barley plants grown in sand culture is shown in Table 7. The deficient plants received one-ninth of the nitrogen given to the controls. Leaves, stems and ears were analysed on three occasions, beginning at the time of emergence of the eighth leaf. The results show the well-known increase in sugar concentration associated with nitrogen deficiency, and further that a large part of this increase is in fructosans. The effect on the ears is much less than that on the leaves and stems.

The effect of potassium and phosphorus deficiency and also of the sodium/calcium balance are shown in Table 8. The sodium/calcium balance is included because of its marked effect on the response to potassium deficiency. These plants were also grown in sand culture, and sampled on three occasions, at the emergence

of the third, sixth and ninth leaves. Potassium deficiency resulted in a lowering both of the fructosan level and of the ratio of fructosan to total sugar, more particularly in the stems. The effect increased with the age of the plant, and was on the whole less under high calcium than under high sodium. This latter effect is attributed to the lowering of assimilation rate, accompanied by an increased respiration and tiller production with the high sodium treatments, resulting in utilisation of most of the available sugar in synthetic processes. In the high calcium treatments vegetative growth is curtailed and assimilation rate little affected, leading to a greater accumulation of soluble sugars.

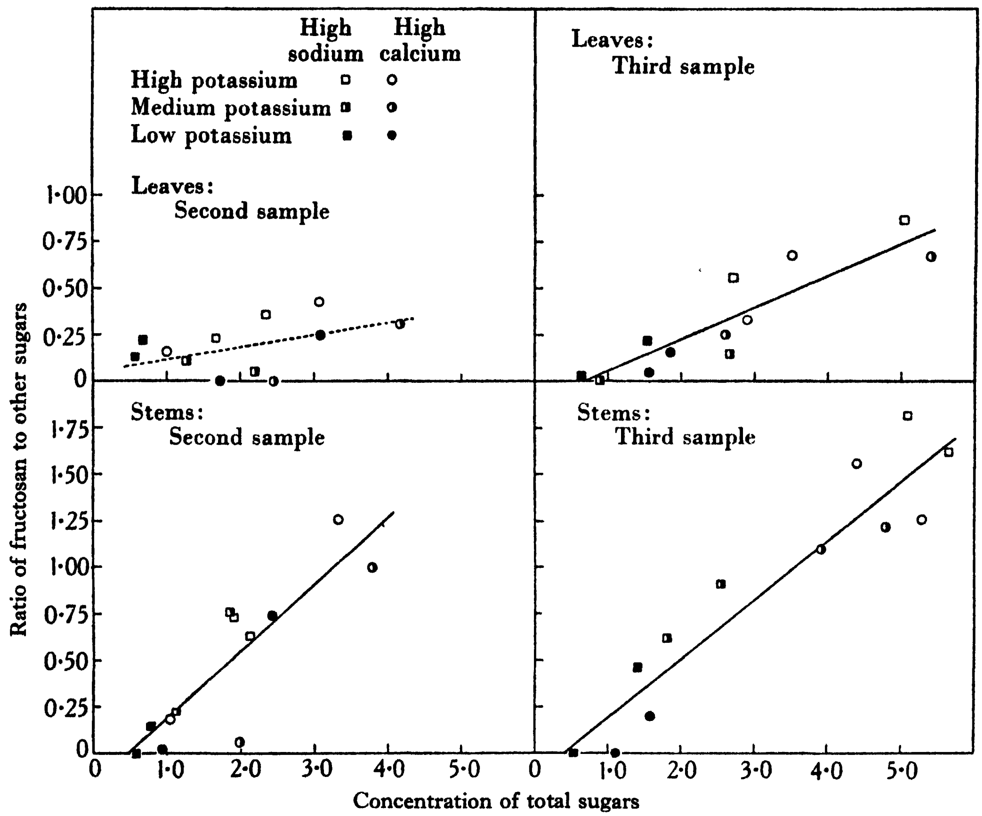

Fig. 7. Regression lines of the ratio fructosan/other sugars on total sugar, in leaves and stems of barley (Plumage Archer) plants grown under various conditions of mineral deficiency. Points are the means of duplicate determinations (from Russell (1938)).

Phosphorus deficiency on the other hand raised both the fructosan level and the ratio of fructosan to total sugar. In this case the effect was greater in leaves than stems, and in the high calcium than in the high sodium treatments. In this type of deficiency vegetative growth is restricted while assimilation rate is less affected, consequently soluble carbohydrates accumulate and particularly fructosan.

It becomes clear from these experiments on manurial deficiencies that fructosans accumulate under all conditions promoting storage of sugar, and further

that high ratios of fructosan to other sugars are associated with high total sugar contents. The question therefore arises as to whether the increased fructosan values are solely due to concentration effects, or whether the manurial treatments themselves have a direct effect. The relationship of total sugar to the ratio of fructosans to other sugars is shown in Fig. 7.

It is evident from the linear nature of the graphs that the ratio must be largely controlled by concentration alone, and it is further clear that there is a characteristic difference between the ratio for stems and for leaves. It has been demonstrated (Russell, 1938) by statistical analysis that age of plant has no direct effect on the ratio, so that the increase with time is solely due to the increasing sugar content. Furthermore, in the stems there was no direct effect of manurial treatment, but in the leaves potassium deficiency lowered the fructosan ratio. This comparatively simple effect of manurial treatment on the partition of sugar between fructosans and other sugars is in marked contrast with the complex interactions which affect the concentration itself and suggests that stored sugars tend to equilibria which are relatively unaffected by manurial deficiency.

9. Effect of tiller and ear removal on fructosan content of barley

If now sugar accumulation is induced by means other than manurial deficiency, namely by mutilating the plant, a similar result as regards the relationship of fructosan to total sugars is achieved. Archbold removed some tillers from one group of barley plants at the time of ear emergence, and from another both tillers and the ears from the remaining tillers. There was an immediate increase of total sugar and fructosan in all parts of the plant in both groups. In the first group the downward trend of sugar in the leaves was arrested and sucrose and fructosans began to accumulate. Fructosan concentration rose from about 0·8 to 1·77 % or 40 % of the total sugar. Additional ear removal increased the fructosan content yet a little more. In the stems the effect was much more marked, the fall in sugar content as the ears developed was prevented and sugar continued to accumulate. When ears also were removed a further increase occurred. Removal of ears also resulted in cessation of stem growth, and accelerated the rate at which the leaves died off, so that finally the plants from which only tillers were removed reached the same high level of sugar as those from which the ears also were removed. The effect of ear and tiller removal as compared with tiller removal only on leaves and stems is shown in Fig. 8.

In considering the results as a whole it may be concluded that any conditions which tend to promote sugar storage result also in the production of relatively large amounts of fructosan. These conditions may be the normal period of sugar storage in the stems, when the leaves deliver more sugar than is immediately required for the growing ears, or they may be abnormal, such as those following tiller or ear removal when the effect is similar but much exaggerated, or they may be due to some mineral deficiency. Fructosan formation therefore, unlike total sugar, depends largely on concentration, and this fact together with the observation that in leaves, the primary seat of synthesis, sucrose accumulates to a considerable extent as well as

fructosan indicates the secondary origin of the fructosan, and its relatively slow rate of formation. Its significance in the general metabolism is thus the same as that of any other soluble sugar, and lies in the fact that in common with them it is a form in which carbohydrate is temporarily stored. To omit fructosans from a quantitative study of sugars may mean disregarding up to half the sugar present.

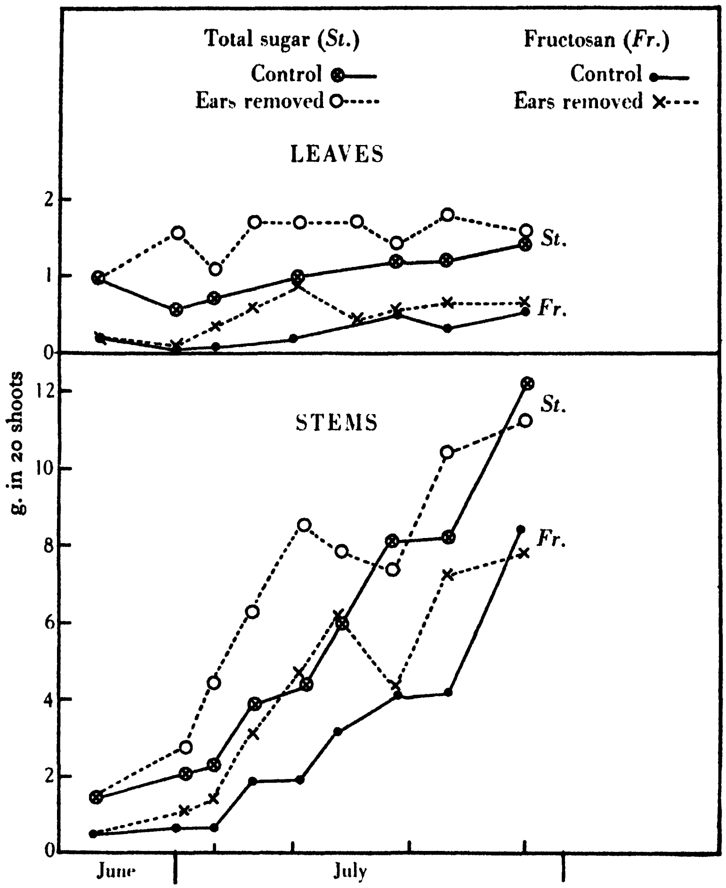

Fig. 8. The effect of ear removal on the total sugar and fructosan contents of leaves and stems of barley (Plumage Archer) (plotted from the data of Archbold (1938)).

The satisfactory solution of the problem of the immediate origin of the fructosans requires information about the whole carbohydrate change in the plant. The possibility that fructose or glucose are preferentially used in synthetic processes or for respiration has been tentatively put forward, but the opinions expressed are often based on consideration of concentration data, a procedure which, it has been pointed out, does not give at all a complete picture of the interrelationships of the carbohydrates, and where actively growing organs are under investigation may be very misleading. Only when it can be stated with certainty under which circumstances glucose and fructose are interconvertible in plants, and when some knowledge has

been acquired of the mechanisms involved both in such conversions and in polymerization, will real progress in our views on fructosan formation be possible.

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THE SUBMERGED FORESTS AT RHYL AND ABERGELE, NORTH WALES

DATA FOR THE STUDY OF POSTGLACIAL HISTORY. III

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(With 3 figures in the text)

CONTENTS

PEAT beds are exposed on the shore along the coast of North Wales in many places and have frequently been described (Neaverson, 1936). The present paper gives some additional facts concerning these deposits at Rhyl and Abergele. Fig. 1 shows the approximate positions of the beds here described, but

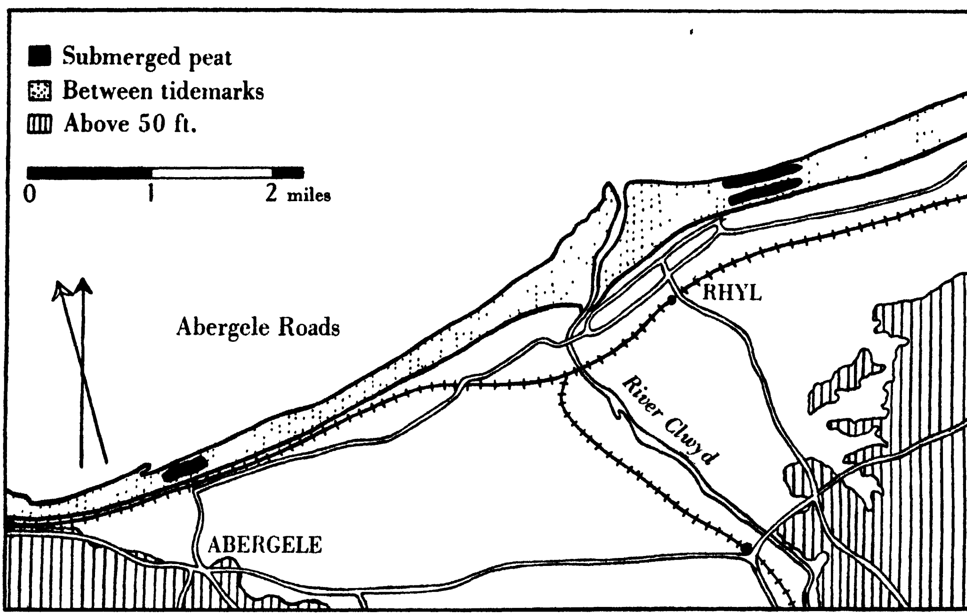

Fig. 1. Submerged forests on part of the North Wales coast.

no attempt is made to map them exactly, since the area of peat exposed varies with the action of the wind and sea.

The Rhyl peat

(1) *Boring*

It is frequently said that the peat consists of an "Upper Forest" and a "Lower Forest". To test this conventional division into Upper and Lower Forests, a boring was taken at the seaward edge of the Rhyl Golf Links, with the results given below. The fourth column gives the letters by which the peaty layers are subsequently known. In the last column is given the author's view of the correspondence between these beds and the exposures usually recognized on the shore.

Depth (ft.)	Thickness of deposit (ft.)	Deposit	Reference Letter	Exposed beds
0– 0·5	0·5	Sand		
0·5– 1·0	0·5	Sandy peat	(*d*)	
1·0– 4·0	3·0	Sand		
4·0– 6·0	2·0	Silty sand		
6·0– 7·0	1·0	Peat	(*c*)	"Upper Bed"
7·0–13·0	6·0	Silt		
13·0–14·0	1·0	Peat	(*b*)	"Lower Bed"
14·0–14·5	0·5	Peaty silt	(*b*)	"Lower Bed"
14·5–15·2	0·7	Silt		"Lower Bed"
15·2–15·7	0·5	Peaty silt	(*a*)	"Lower Bed"
15·7–	?	Silt		

(2) *Direction of trees*

It has often been reported that tree trunks in particular peat deposits lie generally in some specified direction. The numerous trees present in exposures both of the "Lower" and of the "Upper" beds on the shore allowed a closer investigation of this point. The directions of tree trunks were recorded with the aid of a magnetic compass, separate readings being made in the case of the "Upper" bed (*c*), for trees embedded in the peat and for trees lying free on the surface of the peat. In the case of each bed a note was made of those trees whose sense could be determined as well as their direction, the sense being deduced sometimes from the varying width along the trunk, sometimes from the angle of branching, and sometimes from proximity to a stump. In these cases the directional reading was that of the apex of the tree. In other cases a reading of θ° west of north might equally well be $(180+\theta)^\circ$ west of north, and both possibilities are included in the graphical representation. The results are shown graphically in Fig. 2, a line being drawn to represent each tree, in the direction of the tree. Separate diagrams represent those trees of which the sense as well as the direction could be determined.

The diagrams representing merely direction give little or no information, but those representing sense as well as direction are illuminating. In bed (*c*) the general lie of the trunks is definitely in the south-western semicircle, while in bed (*b*) the general lie is to the north-east. This might possibly indicate that the prevailing winds were from the north-east and south-west respectively, during the periods of growth of the two beds.

(3) *Plant content*

Many of the stools lying in and on the peat are clearly of *Quercus* and *Betula*, and many nuts of *Corylus* are also found. Small fragments of wood were also identified microscopically as *Alnus*. The seeds found in the peat, of which the most common is *Rubus*, are listed by Neaverson (1936). The commonest plant fragment was rootlets of a cyperaceous-graminaceous nature, with root-hairs of up to about 1 mm. in length. Fragments of leaf epidermis are common. Much of the material contained spores and hyphae resembling those of the Dematiaceae, and many of the root fragments were highly infected with another fungus closely resembling *Physoderma menyanthis*.

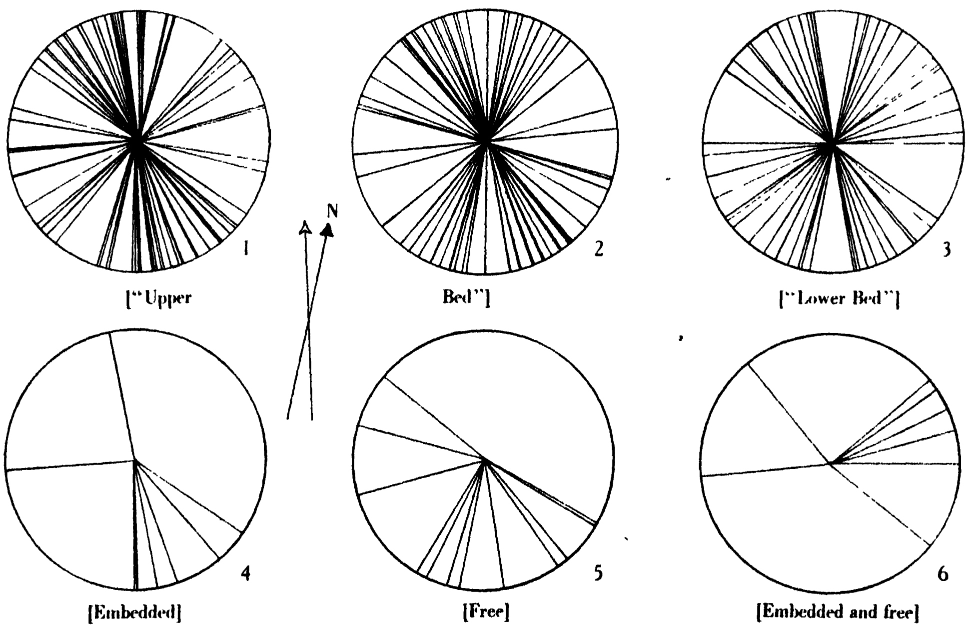

Fig. 2. Graphical representation of directions of tree trunks on Rhyl foreshore. 1–3 show directions only; 4–6 show both directions and sense.

(4) *Pollen analysis*

Samples from beds exposed on the shore. Samples were taken from the upper exposed bed, and the results are given in Table I. Despite the extreme paucity of grains in these peats, it is clear that *Alnus* is overwhelmingly the dominant tree grain, and that *Tilia* and *Pinus* are fairly well represented. A surprising feature is the absence of any important percentage of *Betula* grains, since the wood of this tree occurs frequently on the surface of the peat. By far the most abundant herb grains are those of Gramineae, and spores of *Polypodium* are frequent.

Table I

Depth (in.) ...	1	3	6	7
Alnus	178	25	23	50
Betula	2	1	0	0
Pinus	0	0	3	2
Quercus	1	0	0	0
Tilia	3	0	1	5
Quercetum mixtum	4	0	1	5
Total trees	184	26	27	57
Corylus	4	0	2	1
Trees + shrubs	188	26	29	58
Gramineae	15	4	4	8
Other herbs	2	1	3	4
Total herbs	17	5	7	12
Polypodium	9	4	0	9
(Trees + shrubs)/herbs	11·1	5·2	4·1	4·8

Samples from boring. The results of pollen analysis of the samples taken from the boring are shown diagrammatically in Fig. 3. Evidently the expected *Betula* grains are concentrated in the top of bed (*c*) and this layer is completely eroded from the exposed beds. Grains which were unknown but not unrecognizable, are assumed to be herbaceous. Spores are calculated as a percentage of the total number of herb grains, but are excluded from this total. The ratio of total number of (tree + shrub) grains to the total number of herb grains was calculated, and is assumed to represent density of tree cover. Only those samples are included in the diagram which gave a sufficient number of grains for some reliance to be placed on the calculated percentages.

At the second level in bed (*b*), there is a maximum of *Pinus* and *Quercus*, which may indicate that the peat became drier and that *Pinus* and *Quercus* partially replaced *Alnus* locally and temporarily, until renewed wetness led once more to dominance by *Alnus*. Another possible explanation is that some factor led to a thinning of the *Alnus* cover, with a consequent increase of the relative importance of *Pinus* and *Quercus* grains from a distance. This, however, seems to be less likely in view of the maintained values for Tree Cover and the stools found *in situ*. Bed (*a*) is separated from (*b*) only locally, but appears to have been formed under rather drier conditions, as indicated by the low values for Gramineae, Cyperaceae and Nymphaceae, and the low value of *Alnus*. In the case of bed (*c*) it is fairly clear that an *Alnus* swamp was gradually consolidated, and invaded by *Betula*. This suggestion is supported by the curves for Tree Cover and Spores, which correspond with those of *Betula*. Bed (*c*) does not show the chenopodiaceous grains of the lower beds, possibly indicating less saline conditions. In bed (*d*) the most frequent grains are those of *Salix* and Nymphaceae. It seems fairly clear that the peat was formed under conditions such as prevail to-day in the "slacks" of the Lancashire sand hills.

As regards the age of the beds, it is impossible in the absence of continuous series of pollen analyses from the neighbourhood to serve as reference, to say more than that the beds are post-Boreal and that the *Pinus* and *Tilia* percentages point to a sub-Boreal or Atlantic date for beds (*a*) and (*b*).

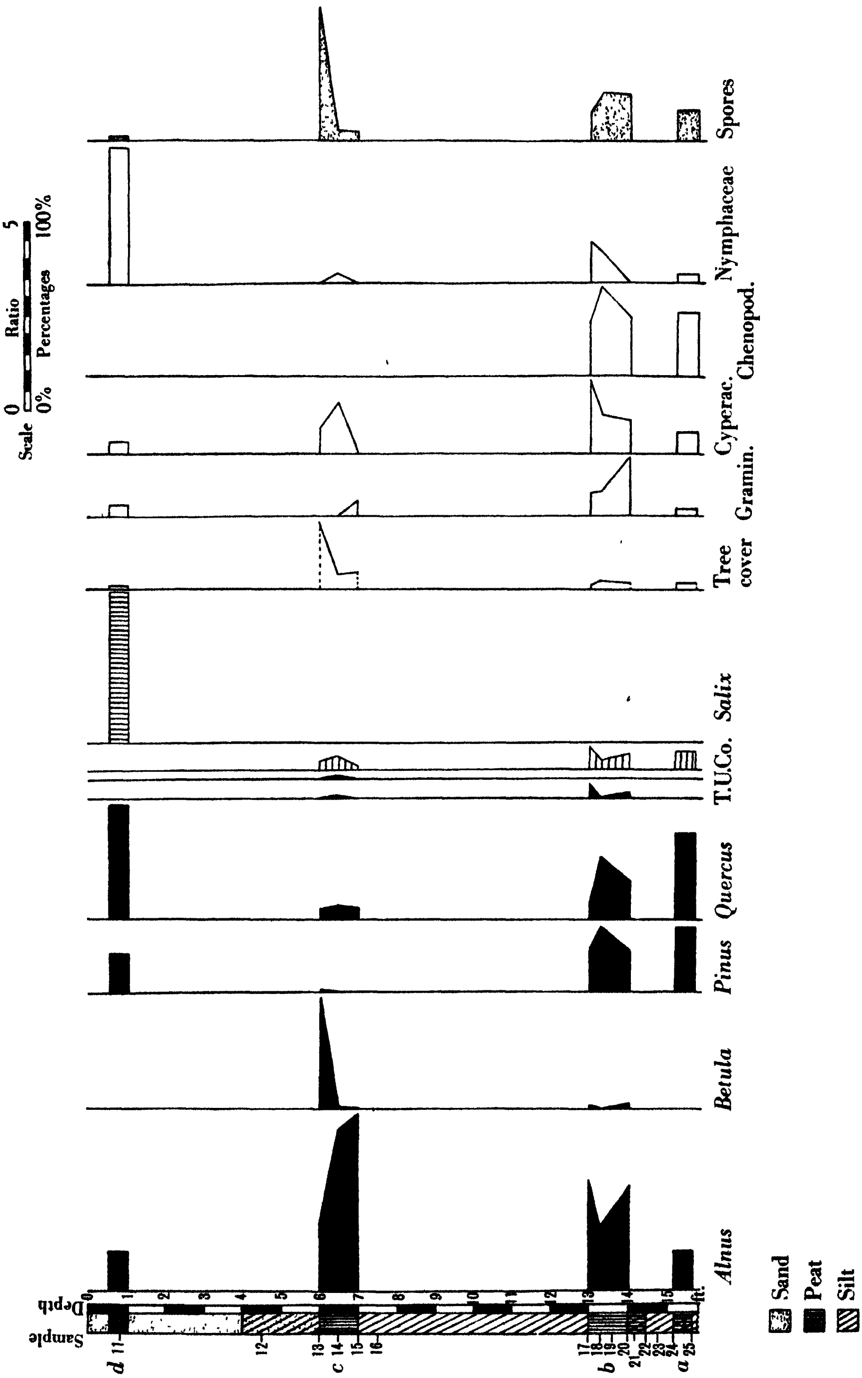

Fig. 3. Pollen analyses of Rhyl foreshore boring.

The Abergele peat

This peat consisted very largely of leaves similar to those of *Phragmites communis*, containing in many cases a fungus closely resembling *Nowakowskiella ramosa*. The peat was very poor in pollen even by comparison with those of Rhyl, but the grains that were identified are listed in Table II.

Table II

Alnus	12	Gramineae	36
Betula	1	Cyperaceae	26
Pinus	5	Compositae	3
Quercus	14	Chenopodiaceae	4
Tilia	7	Nymphaceae	34
Ulmus	1	Polygonaceae	1
Quercetum mixtum	22	Other herbs	8
Total trees	40	Total herbs	112
Corylus	24	*Polypodium*	6
Trees + shrubs	64	(Trees + shrubs)/herbs	0·6

The *Tilia* and *Pinus* counts once again make a sub-Boreal or Atlantic age probable.

The author wishes to thank Dr H. Godwin, Prof. F. T. Brooks, Dr E. Neaverson and Mr W. Mathias for advice on several points, and gratefully to acknowledge the laboratory facilities provided by the University of Liverpool.

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ON A PEAT BOG AT CRAIG-Y-LLYN, GLAM.

DATA FOR THE STUDY OF POST-GLACIAL HISTORY. IV

By H. A. HYDE
National Museum of Wales, Cardiff

(With 4 figures in the text)

The small bog marked on the 6 in. O.S. map (Glam. X, S.E.) as Ffos Ton Cenglau (cf. Fig. 1) is situated at an altitude of *c.* 1600 ft. (=488 m.) near the northern edge of the Glamorgan plateau and about ¾ mile (=1·2 km.) east-south-east of its highest point (Craig-y-llyn, 1969 ft. = 600 m.). The bog is about 550 yd. (500 m.) long measured from north-west to south-east; it attains its maximum width (200 yd. = 180 m.) toward the southern end, whence it narrows rapidly to the north. It is drained from the southern end by a small stream which joins with others to form the head-waters of the Rhondda River. A rapid reconnaissance of the vegetation shows that the dominant plant over the greater part of the bog is *Eriophorum angustifolium*, with which are associated the following species:

Molinia coerulea	f.–l.d.	*Sphagnum recurvum* var. *robustum**	generally distributed
Juncus conglomeratus	o.–a.	*Gymnocolea inflata*	generally distributed
Carex sp. in peaty pools		*Polytrichum commune*	o.
Narthecium ossifragum	v.r.		
Menyanthes trifoliata in pools	r.		

The *Molinia* in general is sparsely distributed, but it forms occasional pure patches in the middle of the bog, and in hummocky form it is dominant along the margins, where in the north-eastern bog it is associated with the *Juncus*, and cotton grass is absent. The banks of the streamlet also are dominated by *Molinia-Juncus*.

The surrounding moorland is a Molinietum in which only the following other species were noted:

Galium saxatile	o.	*Vaccinium Myrtillus*	r.
Juncus squarrosus	o.	*Polytrichum commune*	o.
Potentilla erecta	o.		

Trial borings were made and levels taken along a line (the position of which is approximately indicated as *AB* in Fig. 1). The profile so determined is illustrated in Fig. 2, which shows that the floor of the depression slopes much more rapidly on the west than on the east side. Except where this slope is greatest the peat is underlain by a layer of "clay", which at one point the borer failed to penetrate. The middle of the depression is occupied by fen peat which is succeeded in an upward

* Very kindly identified for me by Mr W. R. Sherrin.

direction by a highly humified peat containing abundant wood remains and, in the main, composed presumably of *Sphagnum*. Throughout the profile the upper horizons are occupied by a *Sphagnum* peat which contains abundant *Eriophorum*

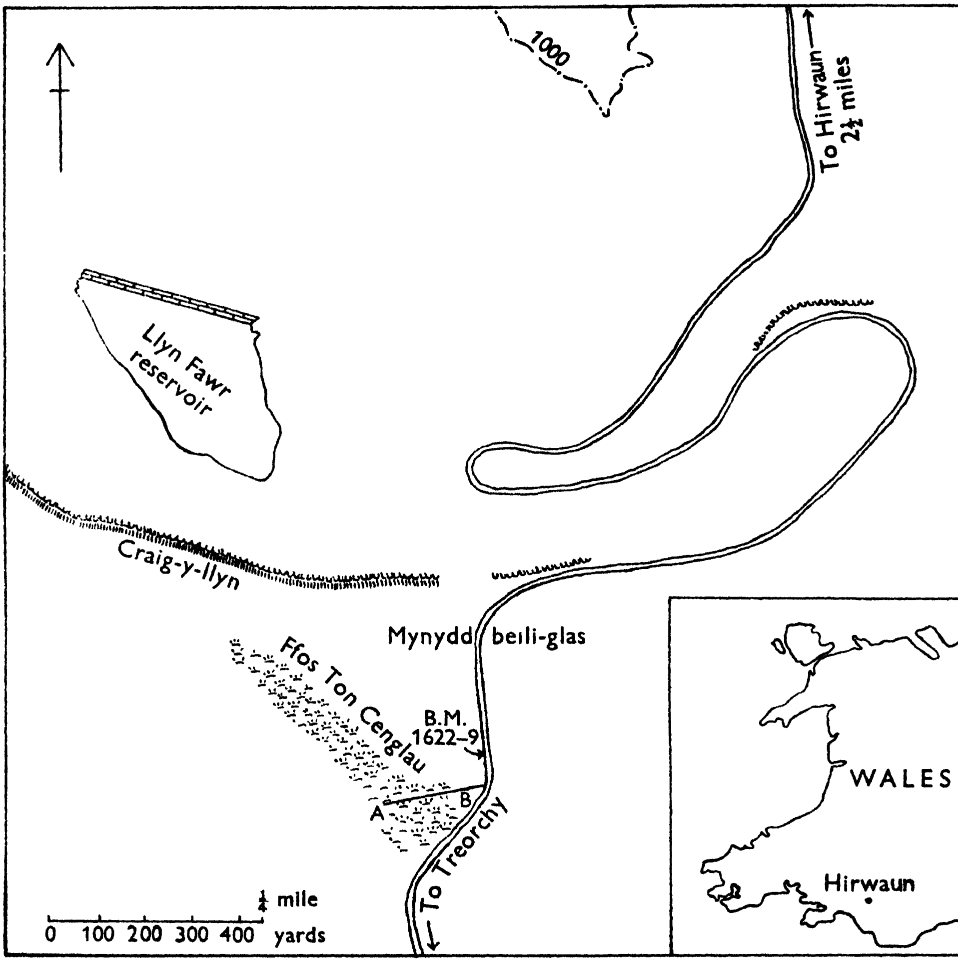

Fig. 1. Sketch-map showing the position of the bog Ffos Ton Cenglau in relation to the northern edge of the Glamorgan plateau and the head-waters of the Rhondda River.

fibre, and from which wood remains are absent. The humification of this upper *Sphagnum* peat varies somewhat, but the transition downward from it to the underlying stratum is readily observable, as the following field observations exemplify:

Bore-hole III:

240–250 cm. Somewhat fibrous brown peat. H. 6–7.*
250–260 cm. Dark brown peat without fibre. H. 9.
260–270 cm. Dark brown peat: first appearance of wood.

* The degree of humification is expressed in terms of Von Post's scale in which H. 1 stands for entirely fresh and H. 10 for completely humified peat.

Bore-hole V:

300–310 cm. Brown peat with considerable fibre. H. 6.
310–320 cm. Brown peat with very much fibre. H. 5. First appearance of wood.
320–330 cm. Much less fibre. H. 6–7.
330–340 cm. Dark brown peat practically without fibre. H. 8. Twig of birch.
340–350 cm. Dark brown peat. H. 9.

Bore-hole VI:

190–200 cm. Brown peat. H. 6–7. Twig (first appearance of wood).
200–210 cm. Brown structureless peat. H. 9.
210–220 cm. Dark brown peat. H. 9. Wood.

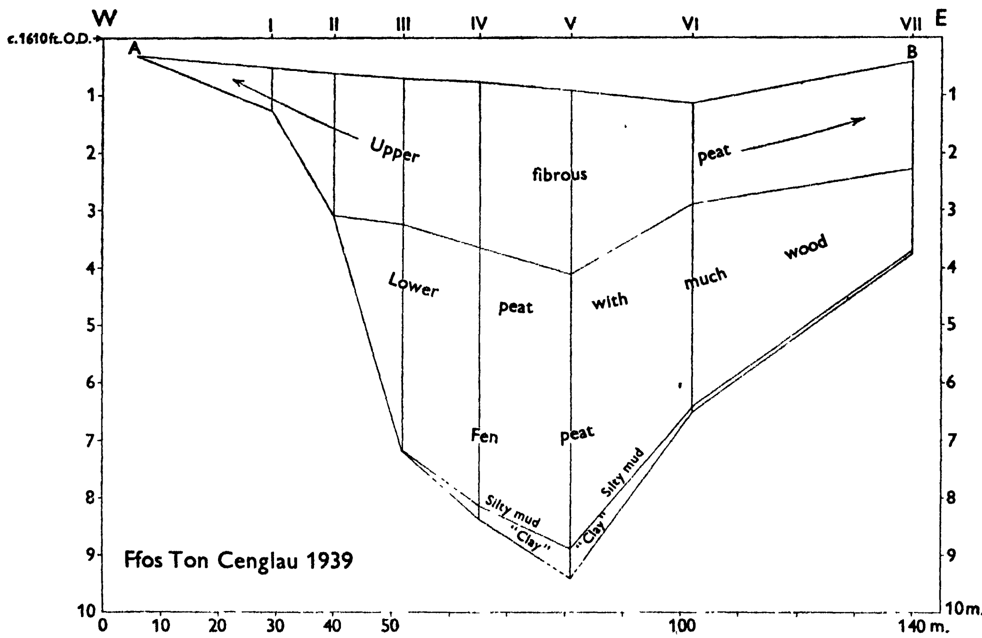

Fig. 2. Section along the line *AB* marked in Fig. 1. The "Grenz Horizont" occurs between the upper fibrous peat and the lower non-fibrous peat.

A complete series of peat samples was taken at a point near the line *AB* where the peat was almost 7 m. deep. Here the profile was as follows:

Ffos Ton Cenglau: Peat profile. 30 *August* 1938

0– 70 cm. Water.
70–150 cm. Light brown *Sphagnum* peat with much *Eriophorum* fibre. Slightly humified (H. 3–5).
150–200 cm. Brownish-black peat with *Eriophorum* fibre more highly humidified (H. 7).
200–210 cm. Brown peat. Less humidified (H. 5).

All the above peat contained a significant proportion of silt, presumably carried into the bog as rain-wash. Below this level silt was absent from the peat samples.

210–385 cm. Bog moss (*Sphagnum*) peat with varying (usually large) quantities of wood, mainly birch (*Betula*) largely in the form of twigs, but some large stems, including one which at 335 cm. prevented the drill-chamber from closing. Humification high throughout, especially at the top (H. 9).

385–495 cm. Brown peat with swamp plants, especially reeds (*Phragmites*) in increasing quantities, and occasional pieces of wood.

495–572 cm. Dark brown peat with much fibre: occasional seeds (? *Menyanthes*).

All the peat from 210 cm. down to this level was practically free from silt.

572–615 cm. Greyish brown mud peat: very fibrous. Some silt in lower 15 cm.

615–645 cm. Dark greyish brown to blackish silty mud: some fibres.

645 cm. + "Clay."

The samples obtained, after being submitted to suitable preliminary treatment including acetolysis, were pollen analysed, the results being set out in Figs. 3 and 4.*

NON-TREE POLLENS AND SPORES (NTP) (Fig. 3)

Separate counts were made of *Sphagnum* spores, fern spores, pollen grains of water plants, Gramineae, Cyperaceae, and other families (varia), and pollen tetrads of Ericaceae. The results have been expressed throughout as percentages of total tree pollen (i.e. trees excluding *Corylus* and *Salix*). Water plants were found only in the lowest horizons, ceasing altogether at 615 cm. *Myriophyllum alterniflorum* occurred to the extent of 1 % from 640 cm. to 630 cm., thus confirming the conclusion (based on a study of the TP curves *vide infra*) that the lowest zone of the profile was of pre-Boreal age.

Sphagnum spores are present in significant quantity from 560 cm. upwards and attain high values (alternating with low ones) at six horizons between 390 and 210 cm. These extreme fluctuations recall the cyclic change in bog development which takes place in the regeneration complex of raised bogs (cf. Godwin & Conway, 1939) and suggest that some such conditions may have become established at Ffos Ton Cenglau. If so, they ceased to obtain from above 210 cm. onwards, from which level upwards *Sphagnum* spores are present in consistently lower numbers.

The changes in the *Sphagnum* curve have their parallels or counterparts in the total NTP curve. Here Ericaceae are seen to reach high levels between 350 and 210 cm., the maxima alternating on the whole with those for *Sphagnum*. Above 210 cm. Ericaceae are still represented but they are quite outnumbered by the other non-tree pollens (mostly grasses and sedges).

The most striking feature of the NTP curves, therefore, is the abruptness of the change at 210 cm. It has already been suggested that this change was due to a sudden increase in rainfall and consequent swamping of the area (Fox & Hyde, 1939).

* The upper parts of these diagrams only (zones VII*b* and VIII) have been published previously (Fox & Hyde, 1939).

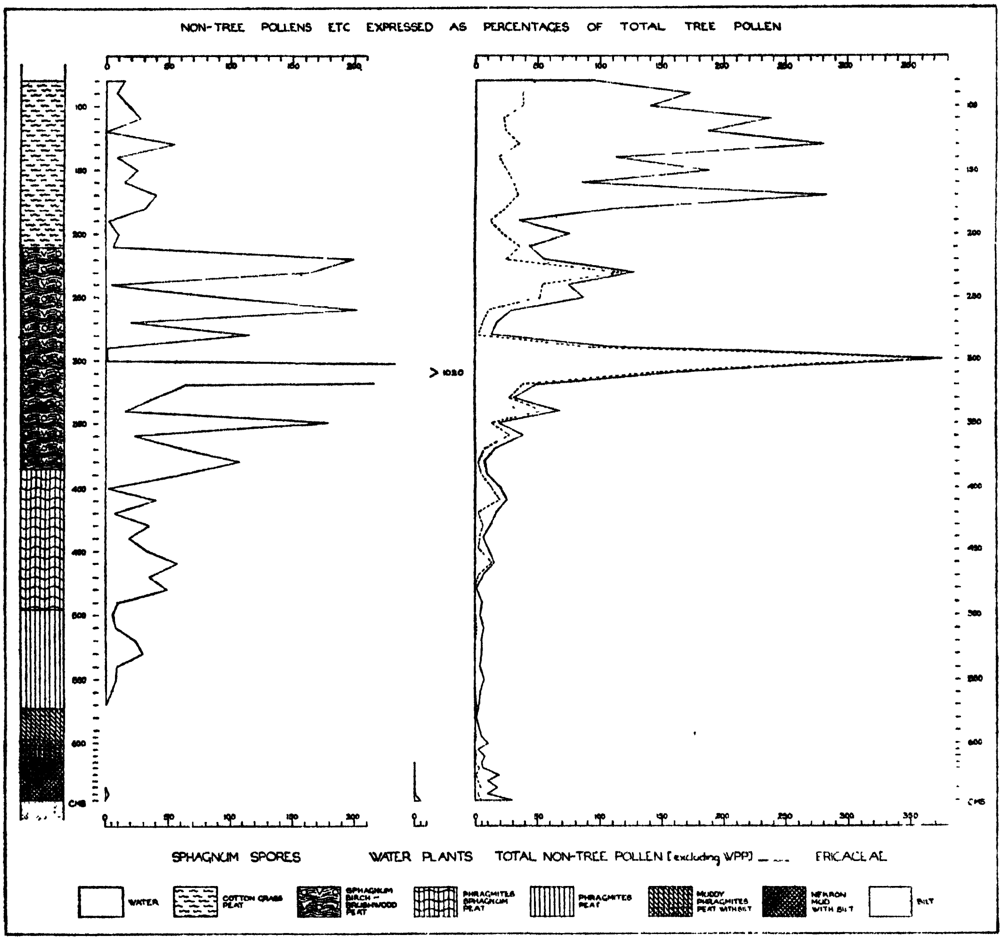

Fig. 3. Non-tree pollen diagram from Ffos Ton Cenglau. For explanation see text.

Tree pollens (TP) (Fig. 4)

The tree pollen analyses have been calculated in the customary manner, i.e. total tree pollen has been taken to exclude the shrubby genera *Corylus* and *Salix*, the results obtained for these latter being expressed as percentages of total tree pollen proper.

The TP curves exhibit wide differences in the percentage composition of the pollen content as one passes from bottom to top. In describing these changes it is convenient to follow the zonal nomenclature adopted by A. R. Clapham & B. N. Clapham (1939) without, however, implying exact correlation between their diagram (even so far as it goes) and the present one.

In zone IV (pre-Boreal) birch is dominant and pine the only other tree significantly represented. Hazel is present in small quantity only. There follows in zone V (transition) a rapid reversal in the relative abundance of pine and birch,

while elm (already present though in minute quantity in zone IV) increases, as does oak, though less rapidly, and hazel rises very markedly.

The succeeding zone (VI) (Boreal) is characterized by the continuing importance of birch and pine. Three phases may be distinguished: in the first (VI*a*) elm is relatively abundant and hazel increasing rapidly; in the second (VI*b*) oak rises

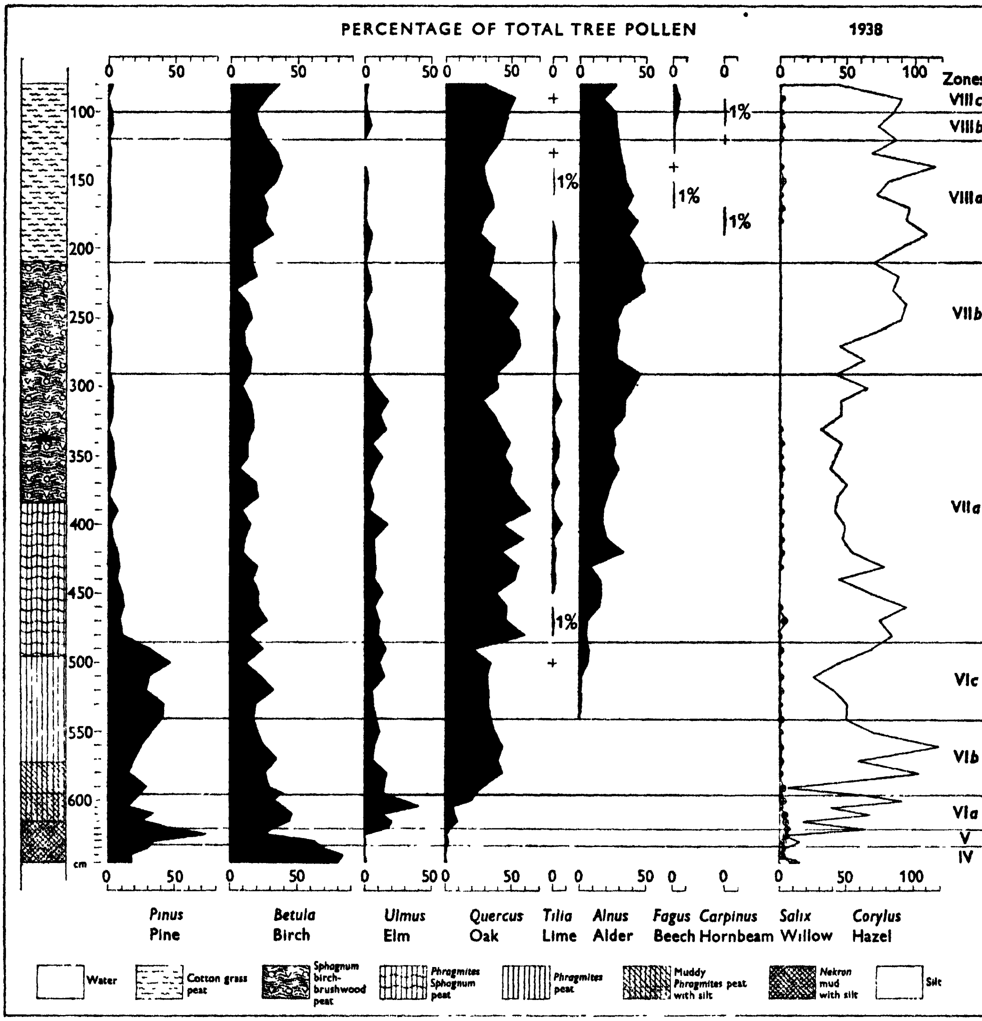

Fig. 4. Tree pollen diagram from Ffos Ton Cenglau. For explanation see text.

rapidly to importance while elm falls and hazel reaches its highest value (120 %); while in the third (VI*c*) pine attains a second maximum, lime and alder appear and hazel now falls rapidly. From the top of zone VI onwards pine declines rapidly and alder increases: this is the Boreal Atlantic transition, which has been observed repeatedly in pollen diagrams from all over north-west Europe.

Zone VII (Atlantic and sub-Boreal) is marked throughout by the relative abundance of mixed oakwood pollen (elm, oak and lime); in particular lime could almost be described as characteristic. Two phases may be recognized: VII*a* with relatively high elm and low hazel, and VII*b* with markedly lower elm (and also pine) and higher hazel. Zone VIII (sub-Atlantic) is characterized by a marked rise in birch and by the highest hazel values above VI*b*. Here again three phases are distinguishable: the first (VIII*a*) sees the virtual disappearance of lime and also the earliest indications of beech; the second (VIII*b*) is marked by a temporary rise in elm and an almost contemporary fall in birch; while in the third (VIII*c*) beech becomes significant.

Discussion

Ffos Ton Cenglau evidently provides at least some kind of reflex of vegetational development from the pre-Boreal period onwards: to what extent this record has been affected by purely local influences remains to be seen. It may be said, however, that, being high up on the Glamorgan plateau, Ffos Ton Cenglau was not in all probability surrounded by woods until well into the Boreal period and, even when it was so surrounded, the pollen rain from the plateau woods in general must have swamped the effect of the trees which grew on the actual bog surface. Wood fragments cease abruptly at the top of zone VII and it is very tempting to suppose that this horizon marks the general disappearance of the upland woods. If so, in zone VIII the curves can only be due to regional and not at all to "local" pollen.

It may therefore be suggested that the Ffos Ton Cenglau diagram will prove to give a fair picture of the post-glacial variations in woodland dominance in South Wales.

The present series of curves appears to be closely comparable with two other sets obtained in this country, namely those relating to Tregaron Bog, Cardiganshire, published by Godwin & Mitchell (1938) and those from the Shropshire and Flint Maelor Mosses recently described by Mrs Megaw (Miss E. M. Hardy, 1939). The zonation of the diagrams from the three areas may be correlated very tentatively as follows:

Tregaron	Ffos Ton Cenglau	Shropshire and Flint	Blytt-Sernander periods
J	VIII*c*		
H	VIII*b*	VIII / ?VII–VIII	Sub-Atlantic
G	VIII*a*		
F_2	VII*b*	VII	Sub-Boreal
F_1	VII*a*		Atlantic
E_3	VI*c*	VI*c*	
E_2	VI*b*	VI*b*	Boreal
E_1	VI*a*	VI*a*	
D	V	V	(Transition)
	IV	IV	Pre-Boreal

Of the three diagrams from Tregaron the one which most closely resembles the present one from Ffos Ton Cenglau is that (constructed by the writer) from Tregaron S.E. 10: both appear to cover the entire period from the pre-Boreal to the present time.

Godwin & Mitchell drew the zonal boundary F_2/G at a horizon where there was a marked change in the nature of the peat from an older, darker and more

highly humified peat to a younger, lighter and less humified one. This horizon they identified with the border horizon (Grenz Horizont) of continental workers. Comparison between the sets of (TP) curves from the two stations leaves little room for doubt that some horizon between 200 and 220 cm. (Fig. 4) should correspond with the F_2/G boundary at Tregaron. The facts already stated in regard to the naked-eye appearance of the profile and the NTP content of the peat indicate clearly that this boundary must be drawn at 210 cm., which is therefore to be recognized as the border horizon. This conclusion is strengthened by observations made when another peat bed was cut through during the making of the Inter-Valley road from Rhigos to Treherbert in 1928. Photographs of the peat sections then uncovered have already been published (Fox & Hyde, 1939).

Certain broad resemblances between the present diagram and Mrs Megaw's are immediately obvious, viz.:

(*a*) The generally concave shape of the birch curve as compared with the convex shape of that for mixed oak-wood.

(*b*) The virtual coincidence of the continuous *Tilia* curve with the phase of more favourable climate hypothecated by von Post (1930).

(*c*) The general course of the elm curve with its marked maxima and minima.

Further and more detailed comparison, though inviting, would probably be premature.

Summary

Pollen analyses made from a small peat bog situated on Craig-y-llyn, Glamorgan, are described; the zonation of the diagram so obtained is briefly discussed and it is shown that the "Grenz Horizont" (border horizon) may be recognized in the bog.

I wish to record with gratitude my indebtedness to Dr H. Godwin for advice in regard to pollen analysis in general and, in particular, for help in zoning the tree pollen diagram. I desire also to thank Mr J. W. Davies who assisted me in the field and the laboratory and also drew the diagrams.

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REVIEWS

Intermediate Botany. By L. J. F. BRIMBLE. $7\frac{1}{2} \times 5$ in. Pp. 562, with 352 figures in the text. London: Macmillan and Co. 2nd edition. 1939. 8*s.* 6*d.*

Some changes have been made in the text and in the illustrations in preparing a second edition of this book, and it is perhaps a pity that they were not even more extensive. The idea lying behind its presentation of the subject is excellent. The need for a change in the method of teaching Botany to elementary students, especially medical students, must be apparent to many. It is therefore a pity that this "endeavour to present botany as a coherent and developing study, of high value in scientific training" falls short of its ideal by a lack of balance and by its unscientific wording. Examples of the latter may be found for instance in Chapter 1. What can be the meaning of the italicized statement that "nature knows no hard and fast lines of distinction between animals and plants"? And what indeed can be the "raw elements" from which plants synthesize food? The word raw has many meanings, but element is a technical term to which raw seems a meaningless epithet. One could quote such examples in hundreds.

As regards the lack of balance in presentation, undoubted advances in some important parts of the subject are ignored, as for instance in the description of meiosis and mitosis; whilst irrelevant sections, such as that on heavy water, give a distorted glimpse of new knowledge the biological importance of which, if any, is not yet known.

Lastly, one cannot refrain from pointing out that this book not only fails to train the young reader to think, but even encourages him to be slipshod. "Now living things naturally continue to live during the hours of darkness, therefore they are working more or less all the time, night and day: consequently they respire during the night. In other words respiration never ceases while the organism is living." This cannot presumably be meant as a proof, but the use of "therefore" and "consequently" would suggest it to the inexperienced mind.

The aim, the printing, and the illustrations of this book, leave one wishing that its details were of an equally high standard.

J. L. HARLEY

Encyclopédie Mycologique, XI. *Les Fusarium et Cylindrocarpon de l'Indochine.* By F. BUGNICOURT. $10 \times 6\frac{1}{2}$ in. Pp. 206, 4 coloured plates, 6 plates of microphotographs, and 36 figures in the text. Paris: Paul Lechevalier. 1939. 165 fr.

The arrangement of the species of *Fusarium* and *Cylindrocarpon* in this monograph is according to the system of Wollenweber, under whom the author has studied. Each species, variety, and form has been described with great care; and for each, habitat, cultural characteristics, micro- and macro-conidial size, and synonyms are given. The biometric details in particular have been carefully studied, and 500 conidia for several different subcultures on each medium were measured. Five media were used to arrive at the grand mean.

A point one misses, however, in these descriptions is any hint of the biology of the species. None is described as a parasite or saprophyte, and the host is never described as being living or dead; a matter which might be of some importance. Moreover, data on this point must have been collected.

Another criticism of the book lies in the arrangement. The table of forms isolated occurs several times in the text; once under the description of genera, once more in part 1 of "the Habitat" and again in great part in a further list of strains maintained in the type collection at Saïgon. These might have been amalgamated in an appendix which would have been of great value for reference.

One must not, however, be too critical of this valuable addition to the literature, which includes in particular careful description of nine new tropical forms of *Cylindrocarpon*, with Latin diagnoses.

The whole monograph is adequately illustrated with excellent plates and figures.

J. L. HARLEY

Earth's Green Mantle. By S. W. MANGHAM. $7\frac{3}{4} \times 5\frac{1}{4}$ in. Pp. 332 with 40 plates and 42 figures. London: English Universities Press Ltd. 1939. 10*s*. 6*d*.

In this book Professor Mangham has set out to explain to the ordinary reader the story of mankind's "persistent efforts to understand and utilize the almost illimitable wealth of plant life forming the Earth's Green Mantle". This enterprise is one which has long awaited adequate handling in print, so that it is perhaps ungracious to point out the inadequacy of a single volume for the carrying out of such a task. Anyone, undertaking to steer the adult student in his quest for the results of scientific research quickly becomes aware of the double menace of the Scylla of a superficial treatment, lacking needful explanation of implied assumptions, and the Charybdis of a detailed exposition that must, for lack of time or space, fail in breadth of presentation. To steer the middle course is a matter of great perplexity. It is perhaps inevitable that the author in this connexion should seem to incline to the side of superficiality. Yet the botanical interests of adult students in the reviewer's experience seem to centre about four distinct problems—the nature of plant life, the machinery of growth, the origin of food plants and the results of scientific research in relation to food production. It might, therefore, have been better to break with the academic teaching traditions and to have rearranged the relevant facts in relation to these questions that naturally occur to the enquiring layman: this despite the successful attempt to dress the conventional approaches in more attractive garb, such as by the use of "Old Clothes" for the title of the chapter dealing with fossil plants or "Mass Production and New Models" for that describing the essential features of reproduction. In this way more emphasis could have been laid upon those aspects of plant life open to further exploitation by man. The excessive brevity of the treatment of the control of photosynthesis under glass (p. 211) or of the factors governing soil degeneration by the accumulation of raw humus (p. 21) are thus to be regretted. Again, much more consideration might well have been given to the origin of cultivated plants, and to the nature of fermentations.

As to the technique of presentation where the ordinary man is concerned, clarity of style and a precise but vivid use of everyday words must come first. Here the author is often very happy in his writing. For instance, in relation to the definition of a plant he says, "Actually it is by no means easy to say exactly what a plant is. Just try to write down a definition of a plant, and if you happen to have a botanical friend ask for a criticism of your definition! There seems but little in common between a speck of yeast, a tangle of pond scum, a mushroom, a seaweed, a moss, a fern, a rose or lily and a giant eucalyptus or redwood tree. Yet your botanical friend will assure you that they are all plants, and you will certainly agree that at any rate they are not animals!" Or again, in connexion with the surface-volume ratio, "Imagine the increase in surface area which would result from cutting a mature pig into rashers as thin as an ordinary foliage leaf."

The careful use of analogy—particularly of analogies with mechanical structures—is of great service in this sort of book, and the author has made good use of it, as will be seen from a glance at the chapter headings. Nevertheless, the energy relations of growth require rather more stressing than they here receive. It is also surely unwise to refer to enzymes as acting "rather like lubricants" unless more is said concerning the mechanism of their action.

The gropings of the individual often recall those of the pioneers, wherefore an historical approach can also be very helpful, as it is in this present work, if not overburdened with detail.

But the final assistance must always lie with adequate illustrations. With these *Earth's Green Mantle* is richly supplied. It is, however, regrettable that many of the plates should have had to be made up on rather a small scale and also that their order should not be related to the accompanying text: some plate photographs do not appear to be referred to in the text. The line drawings are often unimaginative and too geometrical, in the fashion of illustrations of chemical apparatus. Many of these also require greater explanation in the text than they receive. The difficulty of Latin nomenclature has been admirably solved by the use of footnotes that in no way interrupt the smooth reading of the text, whilst the explanation of their significance in terms of the telephone directory is also most happy. The format of this book is excellent and likely to commend it to the casual reader, who may thus be enticed to more continuous study.

M. H. CLIFFORD

Experiments in Plant Physiology. By W. E. LOOMIS and C. A. SHULL. 9×6 in. Pp. xiv+213 (including appendix and index), 52 figures. London: McGraw Hill Publishing Co., Ltd., 1939. 12*s*.

The authors state that in writing this book they have revised, with a view to simplifying experiments so that they may be performed in elementary and intermediate practical classes in plant physiology, the subject-matter of the first part of their larger work, *Methods in Plant Physiology*, which was reviewed on p. 187 of *The New Phytologist*, 1937, vol. **36**. The extent of the alterations, which are mainly omissions of some of the more difficult experiments, is indicated by the fact that in the thirteen chapters concerned 187 experiments were described in 227 pages of the larger work, and 167 experiments are described in 202 pages of the book now under review.

What was excellent in the first book (e.g. the chapter on plant nutrients, and the suggestions for experiments on polarity, regeneration, the properties of auxins, etc.) still retains its excellence. Opinions are bound to differ, however, concerning the worth of some of the experiments. For instance, agreement will not be general that the possible utilization of formaldehyde by green plants (experiment 86) is a suitable experiment for elementary students. It has been noted with surprise that no mention is made of the horizontal microscope in the section on the measurement of growth, or of the klinostat in the section on geotropism. M. THOMAS

An Introduction to Botany, with special reference to the structure of the flowering plant. By J. H. PRIESTLEY and LORNA I. SCOTT. 9×6 in. Pp. 615, 170 text-figs. London: Longmans, Green and Co., 1938. Price 17*s*. 6*d*.

This book is a monument to a remarkable capacity for botanical teaching. Probably Dr Scott, the second author of this book, would be the first to acknowledge the remarkable manner in which Prof. Priestley has succeeded in training along his own individualistic line, a long succession of enthusiastic and able students. The freshness of outlook and capacity for arousing keen interest in botanical science among students can now be shared very widely through a text-book which has lost none of this vitality and stimulus in the writing. The book is chiefly concerned with the structure and biology of the angiospermous plant, which many competent botanists agree to be the best introduction for University students to the subject as a whole. Particular attention is given to the anatomy and development of the monocotyledonous shoot, and few botanists of any standing could read the authors' account of this without pleasure and gain.

The book follows the simplifying system of describing one by one the results of detailed examination of specific plant types. By this means the dangers of faulty generalization are evaded, but in teaching from the book it should be remembered that students will take these particular truths for general ones unless they are given the wider information. The system employed has of course the great merit of encouraging accurate and detailed observation. The authors have not limited their anatomical studies to the text-book types familiar to all botanists, nor to familiar methods of handling the material. Thus, for example, a maceration technique is very effectively employed to demonstrate leaf structure in the small evergreen leaves of *Buxus*.

What made the old subject of physiological anatomy so tiresome was the apparently inescapable tendency towards teleology and argument from design to theoretical function. The authors of the book avoid this, and instead seek to explain structure in terms of developmental history. A particular instance of this is a treatment of the subject of phyllotaxis much more interesting and instructive to students than that found in the standard texts. Naturally, since causal morphology is a subject as yet little developed and

difficult to pursue effectively, there is little accurately known of the deeper causes underlying the differentiation and behaviour of cells, tissues, and organs, and no doubt many will feel that speculation in this field has been excessive. So long, however, as the reader remains critical, the stimulus of the new approach is an advantage outweighing this drawback.

In conclusion it should be said that the illustrations of this book merit ungrudging praise. Instead of the boring repetition of blocks already familiar to dreariness, practically every one of the figures in the book is an original drawing. Not only therefore are the drawings conformable to one another, but they suit the type admirably. They often illustrate material not hitherto exploited by text-book writers.

This extremely original book is sure of widespread adoption in English-speaking universities, and will have a markedly beneficial effect on the early stages of university teaching in them.

H. GODWIN

The Medieval Fenland. By H. C. DARBY. $9 \times 5\frac{3}{4}$ in. Pp. 200, with 11 plates, 25 maps, etc. Cambridge University Press. 1940. Price 12*s*. 6*d*.

The Draining of the Fens. By H. C. DARBY. $9 \times 5\frac{3}{4}$ in. Pp. 312, with 27 plates, 34 maps, etc. Cambridge University Press. 1940. Price 21*s*.

As any British ecologist investigates more and more closely any native piece of vegetation, he will inevitably find that human control has played, or does play, an unsuspectedly large part. Seldom or never may its effects be neglected. These two books of the Cambridge Studies in Economic History will interest him because they trace, by the instrument of historical geography, the progressive story of the conversion of the wildnesses of the natural Fenland of East Anglia into an intensively cultivated farmland. Even the first volume, *The Medieval Fenland*, leaves, however, a large gap between the Romano-British times in which the sea-ward lying marshland of silt was in part deposited, and in which it was extensively cultivated and perhaps drained, and the Dark Ages. It becomes clear that so long as historical records are available, they reveal struggles of the fenmen to control by dykes and sluices or banks, the waters of the river system. Their increasing success, greatly accelerated since the seventeenth century, has led to the use of drainage pumps of increasing efficiency, so that at the present day very little "undrained" fen remains either on the peat or silt land, and all the ancient fen meres, and most of the ancient fen vegetation, have disappeared.

With these changes Dr Darby traces the details of an economy in which the products of natural fen communities, such as turf, duck, sedge, rushes and eels, give place to agricultural crops, at first woad, hemp, colza and opium, and now sugar-beet, celery, potatoes, bulbs and soft-fruit.

All these profound changes, though offering little help in the study of native vegetation, might, it seems to the reviewer, be exploited in another manner by British ecologists. There is a great network of drains of all sizes, but of very similar character, and *of known age* cutting in all directions through the fenland: both in the terrestrial vegetation of the banks and the aquatic plants of the calcareous waters, this seems to offer ideal opportunity for discovering established rates of spread of new species into fresh country. This might especially apply to the silt land area bordering the Wash, which cannot itself be older than the Iron Age or Romano-British period, and to the islands of Jurassic clay or gravel which poke up through the fen peats as islands, covered till historic times with woodland, and only since then open to colonization by more light-demanding species.

The chapter headings of each book furnish a clear idea of the contents. In the first volume they are: I. Introduction: the Pre-Domesday Fenland; II. Occupations; III. Communications; IV. The Changing Prosperity of the Fenland; V. The Care of Banks and Channels, and lastly bibliography and appendices. Perhaps the most striking single new generalization to emerge from this is the tremendous change in the prosperity of the Fenland between Domesday and the early fourteenth century. In a short two hundred and fifty years

the silt fenland is shown to change from country far inferior to the upland, to land a good deal more prosperous than the upland, and to some degree the peat fens shared this improvement. To what extent climatic change, marine retrogression, and social and economic progress were responsible for this cannot now be said, but it is a feature to be recalled in studies in other fields of investigation, and other parts of this country.

The chapter-headings in the second volume are: I. The Prelude to the Draining (1500–1600); II. The Fen Project, 1600–1663; III. The Consequences of the Draining; IV. The Eighteenth Century: The Age of the Windmill; V. The Nineteenth Century: Triumphs and Difficulties; VI. Epilogue: Conditions after 1900, Appendices, Sources, Bibliography and Index.

The reviewer finds it hard to believe that the peat fens were ever, as a whole, higher than the silt lands in level, for parish boundaries, drainage dykes and fen-margin droves so consistently follow or just reach the contour of +10 to +12 ft. O.D., that this must have represented the edge of the peat fens. This, however, is a very small criticism of a work ably and interestingly written, and illustrated by pictures which recall most vividly the sense of almost terrifying emptiness so familiar to those who know the Fenland, and the accompanying sense of human friendliness in the scattered dykes and farms and sluices.

We should like to ask Dr Darby to consider undertaking a comparative study of the draining of the Netherlands, the marshes of the North-west German coast, and of the Fenland. They are regions of similar and related geological structure, and it would be of great interest to find how much they owe to each other, and what they may still learn from one another. The reclamation of the Zuyder Zee is a story that should appeal to any Fenman, and the vision of the great Fenland geologist, Skertchly, of a fertile Victoria county over the reclaimed Wash, may yet be realized if we are willing enough to spend time and thought on the scientific basis of the project.

H. GODWIN

SOCIETY FOR EXPERIMENTAL BIOLOGY

The Society for Experimental Biology normally holds three conferences each year. Short reports of proceedings of botanical interest appear in this *Journal*. Members of the Society can take the *New Phytologist* at special rates. Further particulars can be obtained from the botanical Secretary, Prof. T. A. Bennet-Clark, University College, Nottingham.

FORTY-SEVENTH CONFERENCE

A session of papers on genetics and heterosis was held under the Chairmanship of Prof. J. B. S. Haldane.

Dr Mather gave an account of work on selection of polygenic characters. The number of hairs on the ventral surface of the fourth and fifth abdominal segments of *Drosophila melanogaster* was chosen as the material for investigation. Selection was started in the F_2 of a cross between two stock cultures. Two separate lines involving selection for low and high number of hairs were used. Each line showed advance for two generations and was then stable for the next two generations, but from the fifth to ninth generations a second and more marked advance in response to selection was observed in both high and low lines. Parental stocks used as controls gave little or no advance in either direction. The behaviour of hybrid material was interpreted as showing (*a*) an immediate response due to random recombination of whole chromosomes and followed by stability, (*b*) a later advance due to recombination of genes within chromosomes as a result of crossing-over. This means that chromosomes from the parental stocks had balanced complexes of modifiers of hair number. Crossing-over breaks down and leads to changes in hair number. There is reason to believe that such balanced complexes would develop in the wild state and that there would be a steady slow release of the stored variation by crossing-over which would be available for further adaptation in the event of a change in environment. Linkage thus provides a method of storing potential variation while maintaining comparative stability.

Dr Luckwill gave an account of some recent work on heterosis. He pointed out that, although there is a general tendency for embryos produced by cross-fertilization to be larger than those produced by self-fertilization on the same plant, there is no constant association between increased embryo size and the manifestation of heterosis. When such increases are observed they are not necessarily genetic in origin and are not effective in influencing the final size of the plant. The immediate cause of heterosis is, therefore, considered to be not, as Ashby has supposed, the possession of a larger embryo, but the augmentation of the relative rate of growth at some post-germination stage of the life cycle. This augmentation, however, is often neutralized by the decrease in relative growth rate which results from the possession of a larger embryo, and thus cannot be detected by direct measurement. This theory offers an alternative explanation of Ashby's results.

Mr Nutman described the growth of normal and dwarf grain of rye. The dwarf grain was obtained by immature harvest; ears removed from the plant 5 days after fertilization producing grain one-twentieth the weight of normal grain. The plants which developed from dwarf grain possessed a higher relative growth rate than plants from normal grain: the vigour displayed by the dwarf embryo not being related here to genetic constitution either of embryo or parent embryo sac. Mr Nutman maintained that Ashby's thesis that embryo size determined plant size, cannot be accepted even in a genetically homogeneous population.

Dr Hatcher described experiments concerning the growth of two reciprocal tomato hybrids and of their parent genotypes. Heterosis was manifested during embryo development, but final embryo size was wholly determined by maternal environment. Embryo size had no effect on the size of the plumular organs, and only in the cotyledons was there any evidence of hybrid vigour during purely vegetative growth. With the onset of flowering and fruiting considerable heterosis was established as a result of the combination of favourable characters from both parents.

Prof. Chibnall took the Chair and opened a symposium on nitrogen metabolism of plants.

He discussed the hypothesis of labile protein which may be summarized in the expression protein → amino-acid → carbon residue amino-acid → protein, and pointed out that the protein level was influenced by attachment to the root system, the control of which was possibly hormonic.

In recent work bearing on this problem the isotope N^{15} has been used. Detached mature *Phaseolus* leaves, rooted after treatment with β-indolyl-acetic acid, were supplied with ammonium chloride containing 1·2 % N^{15}. After 30 hours, during a period when there was a nett breakdown of protein to soluble nitrogenous products, the protein of the leaf had acquired N^{15} to the extent of 1·5 % of the protein-N. Schoenheimer and Vickery similarly had shown that leaf protein in buckwheat supplied with a similar source of N^{15} contained this isotope to the extent of about 6 % of the total protein-N and it is therefore clear that a considerable part of the N^{15} is present in the peptide linkages. In discussion it was agreed that this provided almost unmistakable evidence of the opening and closing of peptide linkages and of reversible deamination which was not, however, in itself direct evidence of the protein ⇌ carbon residue equilibrium.

Prof. Pearsall described work on *Narcissus* leaves floating on glucose + ammonium nitrate. Greater nitrogen absorption occurred in light than in darkness; nitrate reduction was accelerated in the green parts of the leaves in light. The fact that light accelerated formation of organic nitrogen compounds but that temperature did not was held to indicate that the effect of light was on the permeability to centres of synthesis. Balance sheets showed that much nitrogen was lost in gaseous form and it was assumed that half of this originated from nitrite and half from amino-groups. In discussion the point was raised that this interaction occurs within a very limited *p*H range which might often preclude its occurrence *in vivo*. Clear evidence on this aspect of the reaction is not yet available.

Dr Steward described work on disks of storage tissue (mostly potato tuber), which, it was pointed out, grow and synthesize protein at the expense of amino-acid. Many variables influence protein synthesis and respiration in the same sense. The contrasting effects of potassium and calcium salts having a common anion is outstanding. Increasing concentrations of potassium salts cause decrease of sugar concentration, increase of protein synthesis, respiration, and of browning of tissues due to *o*-quinone formation which latter may be related to deamination of amino-acids and the supply of nitrogen used in protein synthesis. Increasing calcium salt concentrations have opposite effects. In potato disks the effects of salts on respiration are not due to their effects on sugar concentration but they are consistently paralleled by the effects on protein synthesis. All the ions investigated have specific effects on both respiration and protein synthesis. The results of a large number of salt combinations all tend to confirm the view that the effects of salts operate through the link between protein synthesis and respiration which deamination of amino-acids provides.

Prof. Gregory, speaking of work carried out in his department by Dr Scott Russell, made it clear that previous work on alleged effects of amino-acids on respiration of leaves had to be regarded with considerable caution as the activity of contaminating bacteria was often not eliminated. The method of investigating bacterium-free cultures of *Elodea* was described and it was shown that in the absence of bacteria respiration of *Elodea* was not increased by amino-acids as had been previously claimed.

Dr G. E. Blackman described the continuation of work on nitrogen metabolism of *Agrostis tenuis* and *Festuca rubra*. Complex interaction between effects of light intensity and nitrogen supply of leaves of frequently defoliated plants were observed. With a low nitrogen supply, shaded leaves (0·4 daylight) contain more total protein and nitrate, less sugars, and the same total organic acid content as leaves from plants in full daylight. In daylight, additions of ammonium sulphate or calcium nitrate cause gains of protein, nitrate, and organic acids but loss of sugars. At 0·4 daylight manuring increases total nitrogen but may depress the protein level. This failure to synthesize protein cannot be attributed to lack of sugars or organic acids but rather to some unknown factor.

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OXIDASE SYSTEMS IN THE TISSUES OF THE HIGHER PLANTS

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CONTENTS

I. INTRODUCTION

RESPIRATION is the process by which the cell extracts energy from food materials. The stages by which even the simplest carbohydrate is broken down are as yet imperfectly recognized. It would seem, however, that the aerobic breakdown of hexoses in the higher plants can be divided into two steps, glycolysis and the oxidation of its products. Glycolysis is probably brought about through some hexosephosphate combination. The products of glycolysis are oxidized by a series of oxidation-reduction systems of graded potential up to molecular oxygen. This series of oxidation-reduction systems transfers hydrogen step by step from the oxidizable substrate to molecular oxygen as the final hydrogen acceptor. Each system is oxidized by the reduction of a system of more positive potential ending in the reduction of oxygen.

Each step in this oxidation-reduction chain requires two agents, a protein, or dehydrogenase, and an oxidation-reduction system which acts as a hydrogen transporter. It is purely a matter of nomenclature whether the whole system, comprising a protein carrier and an active (prosthetic) group, is called the oxidase enzyme or whether the protein is described as the enzyme (dehydrogenase) and the oxidation-reduction system as the coenzyme. The relationship between the two parts and the oxidizable substrate is not certain. It is not known whether, as Warburg holds, the protein is no more than the carrier of the prosthetic group or the protein activates the substrate, i.e. so alters the configuration of the substrate that it is ready to exchange electrons with the oxidation-reduction unit. In view of earlier definitions of a coenzyme, the statement that the prosthetic group of an oxidase and the coenzyme are identical and the inference that all enzymes have a coenzyme may appear to be incorrect, as it has been generally stated that, while certain enzymes were active only in the presence of a specific coenzyme, others

required no such accelerator. The real distinction between the enzymes lies, however, between those in which the protein and prosthetic group are easily dissociated and those in which they are firmly bound. It has been found that the protein-pyridine nucleotide enzymes are more easily dissociated than the protein-alloxazin oxidizing systems. In the former case the prosthetic group—pyridine nucleotide—can be removed by washing and has long been described as the coenzyme, while in many of the latter the alloxazin derivative can only be removed by more drastic treatment, such as dilute acid hydrolysis, and many of the enzymes of this group have been described as requiring no coenzyme—for the very good reason that it is firmly attached to the protein.

The activities of the two parts of the oxidizing system may be considered separately. The work which has been done on the constitution of certain enzymes, including xanthine oxidase, *d*-amino acid oxidase and the Warburg "yellow respiration ferment", reveals that while each of these enzymes is specific to a particular substrate the prosthetic group in each is the same, i.e. alloxazin adenine dinucleotide. This suggests that the protein component determines the specificity of the enzyme towards the substrate. Whether this specificity can be expressed in terms of molecular configuration or in the manner in which the protein modifies the potential of the prosthetic group is unknown.

It is proposed to consider the oxidase enzymes present in plant tissue in terms of the prosthetic group which is attached to the protein carrier. There have been isolated from plant tissues a number of substances which have been recognized as relatively simple organic compounds possessed of reversible oxidation-reduction properties, including hydroxynaphthoquinones, Palladin's respiratory chromogens and hermidon. Their part in the respiratory chain is as yet unknown, and it may be that they are the prosthetic group of some very easily dissociated enzyme, if this view is correct their association with a specific protein carrier has yet to be observed.

II. METAL PROTEIN COMPLEXES

The enzymic oxidation of the three substrates, ascorbic acid, dioxymaleic acid and orthodihydroxy compounds, involves basically the linkage of two hydrogen atoms to one of oxygen and their removal as a molecule of water. The nature of the enzyme responsible for activating this process has been considered by Szent-Györgyi and his co-workers (1938). The starting-point of their work was the colour reaction given by a few compounds with a solution of a ferrous salt. They found that only three compounds gave the violet colour with equal intensity on the addition of a ferrous salt to a neutral solution. These compounds were ascorbic and dioxymaleic acids and catechol compounds. The colour only developed in the presence of oxygen and disappeared on the addition of a reducing agent such as sodium hydrosulphite.

The reaction between catechol and ferrous salts was investigated by Weinland & Binder (1912), who concluded that the ferrous ion formed with catechol in neutral solution a colourless complex which in air was rapidly oxidized to a ferric complex,

this latter complex containing in its molecule one ferric ion, two molecules of catechol and one molecule of water. The striking property of this complex compound was its great stability, in other words its slight tendency to dissociate. In neutral solution the complex is practically undissociated.

Szent-Györgyi and his co-workers (1938) reinvestigated this reaction between ferrous iron and catechol. They found that on addition of a neutral solution of catechol to a ferrous salt, autoxidation results. The first stage in the reaction is the dissociation of the catechol molecule as follows:

$$C_6H_4(OH)_2 \rightleftharpoons C_6H_4O_2^{--} + 2H^+.$$

The mobile hydrogen ions are therefore liberated into the solution immediately by dissociation, and the catechol enters the complex as two equal ions. The complex may be represented as follows:

$$2C_6H_4O_2^{--} + Fe^{++} \rightarrow \begin{bmatrix} Fe^{++}(C_6H_4O_2)_2 \\ H_2O \end{bmatrix}^{--}.$$

The two catechol anions combine with one ferrous ion, then oxidation of this ferrous ion in the complex occurs. At this stage there are practically no ferric ions in solution so that the oxidation is not brought about by ferric ions, but it must take place by an intra-molecular electron displacement between the ferrous ion and catechol in the complex.

Laki & Papp (1938) showed that autoxidation of the complex is strongly inhibited by carbon monoxide, and that the degree of inhibition can be expressed by an affinity constant which proves that the first stage of the reaction between the complex and oxygen must pass through a reversible binding of the oxygen molecule. The oxygen molecule is therefore built into the complex as such, this process constituting the "activation" of the oxygen molecule, and proceeding as in the following equation:

$$O_2 + \begin{bmatrix} Fe^{++}(C_6H_4O_2)_2 \\ H_2O \end{bmatrix}^{--} \rightleftharpoons \begin{bmatrix} Fe^{++}(C_6H_4O_2)_2 \\ O_2;\ H_2O \end{bmatrix}^{--}.$$

Electron displacement from ferrous iron to oxygen occurs so that the ferrous iron is oxidized to ferric iron and the oxygen acquires a negative charge. This results in the splitting of the double bond in the oxygen, then the charged oxygen can unite with the hydrogen ions formed by dissociation of the catechol molecule producing water.

Electron displacement from catechol to the ferric iron then takes place and the latter is thereby reduced back to ferrous iron. These two electron displacements are repeated four times so that the two catechol anions lose their charge, the double bonds are formed and two free molecules of orthoquinone are formed. The sum total of the reactions proceeding through one molecule of the complex may therefore be represented:

$$2C_6H_4(OH)_2 + O_2 \rightleftharpoons 2C_6H_4O_2 + 2H_2O.$$

The important property of the complex is the capacity of the catechol to lose its charges thus acting as a reduction medium. The capacity to form an iron complex and thereby to activate the iron is shown by catechol and many other substances, but with most of the latter the reaction ceases at the complex formation,

no electron exchange occurs, thus there is no oxidation of the complex-forming radicle. Only a few compounds show the electron exchange and therefore are autoxidizable.

For biological oxidations the only compounds useful for reversible oxidation are those containing the $\begin{array}{l} \mathrm{HO{-}C{-}}R_1 \\ \quad\ \ \| \\ \mathrm{HO{-}C{-}}R_2 \end{array}$ group. This group is, however, very unstable, and according to Szent-Györgyi (1938), the only other substances which contain this group in stable form are the orthodihydroxy compounds, ascorbic acid and its analogues and dihydroxymaleic acid. This explains why these compounds give the same reaction as catechol with ferrous iron in the presence of oxygen.

Banga (1938*a*) has obtained a similar series of reactions, with only slight differences, between catechol and copper salts in place of ferrous salts. This investigator (1938*b*) has also studied complex formation between iron and ascorbic acid. This complex absorbs oxygen very rapidly, it is less stable than the catechol-iron complex and carbon dioxide may even be evolved. This observation gives some support to the view that the ascorbic acid oxidase system is not an important system for the introduction of oxygen into the respiratory mechanism, for the ascorbic acid complex appears to be too unstable to take part in a cyclic scheme.

The work on the above metal-catalysed oxidations is confined to cases in which the autoxidation is a dehydrogenation, that is, the oxidation of orthodihydroxy compounds, ascorbic acid and dihydroxymaleic acid. It is considered that catechol oxidase and ascorbic acid oxidase are simply acting as copper compounds and oxidizing catechol compounds and ascorbic acid respectively by the mechanism of complex formation, oxygen addition and electron transfer as described. Szent-Györgyi and his co-workers (1938) have shown that a third molecule which may be co-ordinated into the complex can influence the properties of the complex so that the reaction which leads to autoxidation is accelerated. Nitrogen-containing substances seem to be especially suitable for this work and this may be the significance of the protein in catechol oxidase and ascorbic acid oxidase. Banga & Philippot (1939) consider, without any direct evidence however, that dioxymaleic acid oxidase is a metal protein, and it will be of great interest to find whether iron or copper is present in the enzyme molecule. These oxidase enzymes all appear, therefore, to be metal protein complexes in which the active group, either an iron or copper ion, is associated with a protein carrier. If, as would also appear from this work, the enzymes which activate the oxidation of polyphenols, dioxymaleic acid and ascorbic acid are metal proteins which form complex units with these substances it is possible to regard the metal-protein oxidases as consisting of a protein + a very firmly attached metal ion + a very loosely combined prosthetic group or coenzyme. These three coenzymes contain the common oxidation-reduction group:

$$\begin{array}{l} \diagdown \\ \ \mathrm{C{-}OH} \\ \ \| \\ \ \mathrm{C{-}OH} \\ \diagup \end{array}$$

These metal-protein oxidases with their double prosthetic groups would then be

more directly comparable as oxidation-reduction systems with those oxidases which only contain a single prosthetic group and would be more easily recognizable as part of the respiration chain.

While our knowledge of the polyphenol oxidase system is complete enough to permit this view of its constitution to be regarded as probably correct, that of the activities of the ascorbic acid and dioxymaleic acid oxidases is too small to allow their structures to be more than a matter of speculation. This is particularly the case in regard to dioxymaleic acid oxidase, as very recently Swedin & Theorell (1940), having prepared, purified and examined the properties of this enzyme isolated from *Rumex acetosa*, *Fraxinus excelsior*, *Prunus Padus*, *Syringa vulgaris*, horse-radish root and cauliflower, have concluded that there is not sufficient reason for the assumption that dioxymaleic acid oxidase and peroxidase are different ferments. This is clearly a matter of some importance and has yet to be confirmed. It is proposed, however, in view of the uncertainty in this matter, to adhere to the earlier suggestion that these three enzymes are metal proteins and to discuss them as members of this group.

(1) *Polyphenol oxidase system*

Various substances in plant tissues catalyse the oxidation of phenols by oxygen, iron salts have been isolated which show a weak catechol oxidase-like action. Bertrand (1897) prepared a substance from lucerne which contained traces of manganese, but whose activity on addition of manganese was considerably increased. He showed that manganese salts, particularly those of organic acids, would accelerate the autoxidation of quinol and concluded that manganese was the active agent in his lucerne preparation which he called "Lucerne laccase". The manganese was supposed to be combined with some acid radicle, probably a protein. It is important to notice that at this time Bertrand was unaware of the existence of peroxidase as a separate enzyme. It is possible that the manganese compounds, in some, at least, of his enzyme preparations, acted by producing hydrogen peroxide which with peroxidase would cause oxidation of the phenolic substrate. Euler & Bolin (1909) showed that Bertrand's "Lucerne laccase" was a mixture of small amounts of calcium salts of organic acids which in the presence of traces of manganese catalysed the oxidation of polyphenols. They were able to imitate the action successfully and apparently exactly with a mixture of the calcium salts of malic, glycollic and mesoxalic acids. This "Lucerne laccase" is thermostable and cannot therefore be termed a true enzyme.

Wieland & Loevenskiold (1936) obtained from the fungus *Lactarius vellereus* a preparation which was heat labile and which catalysed the autoxidation of hydroquinone to quinone. But their preparation remained active after dialysis through a fish bladder and was therefore of low molecular weight and not a true enzyme.

True enzymes, non-dialysable and thermolabile, capable of catalysing the oxidation of phenolic compounds, are, however, much more widely distributed in plants than are these non-enzymic activators. In addition to catechol oxidase, the following enzymes have the property of catalysing the oxidation of phenols in air:

tyrosinase and indophenol (cytochrome) oxidase. Peroxidase only carries out such oxidations in the presence of hydrogen peroxide, in which property it differs from catechol oxidase. It is unable to catalyse oxidations in the presence of atmospheric oxygen. Catechol oxidase catalyses phenol oxidation in the presence of oxygen but not in the presence of hydrogen peroxide.

Tyrosinase or monophenol oxidase has essentially all the properties of catechol oxidase, and in addition it catalyses the autoxidation of monophenols such as tyrosine. But although guaiacol contains only one free hydroxyl group, it is not attacked by tyrosinase, while catechol oxidase, which is supposedly specific to compounds having the orthodihydroxy grouping, oxidizes guaiacol to tetra-guaiacoquinone. This paradoxical behaviour of tyrosinase and catechol oxidase, and, indeed, the specific substrates of both enzymes, require further investigation. While Richter (1934) suggests that catechol oxidase and tyrosinase are two distinct enzymes, Graubaud & Nelson (1935) conclude that they are one and the same enzyme.

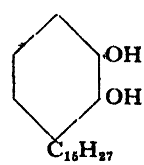
Until the work of Keilin (1929) the enzymic oxidation of phenylenediamine was regarded as being due to catechol oxidase. Keilin was able to show that the reaction is actually catalysed by a separate enzyme, indophenol or cytochrome oxidase, and proved that catechol oxidase from potato tubers can only oxidize phenylenediamine in the presence of catechol or some other orthodihydroxy compound. Sutter (1929) showed that a phenol oxidase preparation from *L. vellereus* also required the presence of an orthodihydroxy compound to catalyse the oxidation of phenylenediamine. The oxidation in these latter two cases is therefore indirect, only proceeding through the orthoquinone primarily formed. We regard the oxidation of orthodihydroxy compounds catalysed by catechol oxidase as a single direct enzyme reaction.

Varying results have been obtained using catechol oxidase preparations from different sources: a 50 % inhibition of potato catechol oxidase is produced by cyanide in a concentration of $5 \times 10^{-4}M$, while the same inhibition is only produced in apple catechol oxidase by $70 \times 10^{-4}M$ cyanide (Wieland & Sutter, 1930). Several different types of catechol oxidase have been described. The laccase obtained from *Rhus succedanea* has recently been investigated by Keilin & Mann (1939), who find that this enzyme is not inhibited by carbon monoxide. Catechol oxidase obtained from other sources, such as potato tubers and mushrooms, is strongly inhibited by carbon monoxide. Laccase when 50 % pure has a blue colour, while catechol oxidase obtained from *Lactarius vellereus* is yellow, and that from potato tubers is greyish. The purest preparations of catechol oxidase from various sources show differences in activity, potato tuber catechol oxidase and laccase show approximately the same activity, but both show only one-twentieth of the activity of the enzyme from the cultivated mushroom. These differences may depend upon differences in the colloidal carrier, the active group, or possibly the associated orthodihydroxy compound. It is essential to state the source of the enzyme preparation used in any work on catechol oxidase, though it is probable that the enzymes from different sources are essentially the same. Kubowitz (1937) found that potato catechol oxidase is a copper-containing protein compound, and Keilin

& Mann (1938) have shown that a catechol oxidase preparation from *Agaricus* when pure also contains copper but no haematin, iron or manganese. The group of enzymes, which catalyse the autoxidation of phenols are known as phenolases. Those which catalyse specifically the autoxidation of orthodihydroxy compounds are best called catechol oxidase although other terms have been used such as polyphenolase, oxygenase, laccase.

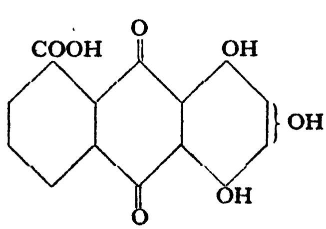
Catechol oxidase not only has the property of catalysing the autoxidation of orthodihydroxy compounds but is also responsible for the coloration of injured plant tissues when exposed to air. The phenomenon is well known in the case of the apple, pear and certain fungi, such as *Boletus*. Yoshida (1883) showed that the darkening in colour of crushed lac tree (*Rhus vernicifera*) stem on exposure to air was caused by an enzyme which he called "laccase". The researches of Yoshida were carried further by Bertrand who found (1896) that the substrate of laccase in Japan lac (*Rhus succedanea*) was a polyphenol—uroshiol—and that the coloration of the crushed lac stem was due to the oxidation of this phenol by atmospheric oxygen. Bertrand also showed that the same enzyme "laccase" catalysed the oxidation of other phenols such as catechol, hydroquinone and pyrogallol (1896).

Polyphenols are widely distributed throughout the plant kingdom. Some of these are responsible for the coloration of injured tissues when exposed to air, as mentioned above, but their constitution is only known in a few cases. Majima (1922) has worked out the constitution of the substrate, uroshiol, of the Japan lac oxidase and found it to be a catechol derivative with the formula



In Tonkin lac (*Rhus vernicifera*) laccol is the enzyme substrate which Bertrand & Brooks (1933*a* and *b*) have shown to be the next higher homologue of uroshiol. The lac formation is brought about first by the enzymic oxidation of the phenol to the orthoquinone, then further oxidation follows, probably without any enzymic action, similar to the enzymic and non-enzymic reactions in melanin formation by tyrosinase as demonstrated by Raper (1926).

In some species of *Boletus* the yellow-orange flesh turns blue on injury. This is particularly striking in the Satan fungus. Bertrand (1901) investigated the red colouring matter of this fungus and named it boletol. Kögl & Deijs (1935) have demonstrated the following formula for boletol:



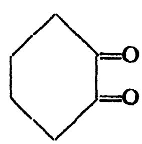
Catechol oxidase oxidizes the para-positioned hydroxyl groups forming the diquinone which gives blue alkali salts, thus bringing about the blue coloration of the injured tissue.

Mechanism of the biological oxidation of catechol

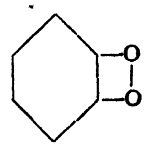
The theory of Bertrand on the nature of the agent in plant tissues capable of oxidizing catechol was followed by that of Bach & Chodat. This theory in its final form gave the name oxygenase to an enzyme capable of oxidizing an associated substrate in the presence of air to a peroxide. From time to time the existence of oxygenase has been questioned and the recorded phenomena were ascribed to the presence of an autoxidizable substance only. It is, however, now clear that there does exist in certain plants an enzyme which is capable of oxidizing either the associated substrate, which contains an orthodihydroxy grouping, or catechol itself to a peroxide or orthoquinone structure. This enzyme is known by several names including Bach & Chodat's original term, oxygenase, polyphenol or catechol oxidases. Onslow stated that this enzyme preparation is always associated with peroxidase, but Sutter (1936) has obtained from certain fungi and from Japan lac preparations of catechol oxidase of great activity perfectly free from peroxidase. Wieland & Sutter (1928) have shown that peroxidase is specific for oxidations involving hydrogen peroxide and cannot activate organic peroxides. This enzyme system, catechol and catechol oxidase, has been found to be capable of carrying out secondary oxidations *in vitro* of a large number of substances including amino-acids. The work of Onslow & Robinson (1928) yields strong evidence that these secondary oxidations are due to the activity of the catechol oxidation product, possibly an orthoquinone. These secondary reactions are probably purely chemical and not enzymic.

Further, Szent-Györgyi (1925) was able to show that the blue colour which developed on the addition of guaiacol to a tissue containing the catechol oxidase system did not require the presence of a peroxidase. The essentials were an orthodihydroxy compound and the catechol oxidase enzyme. The oxidation of the former resulted in a quinone which by the non-enzymic oxidation of guaiacol produced the blue colour. This colour reaction is used as a test for the presence or absence of the catechol oxidase system in any tissue.

There has been some dispute concerning the nature of the oxidation product resulting from the activity of catechol oxidase on catechol. In view of the work of Willstätter & Müller (1908) on the oxidation product of catechol, Szent-Györgyi (1925) considered that the enzymic oxidation of catechol produced the diketoquinone and not the peroxide form, as the latter is extremely labile:



Diketoquinone

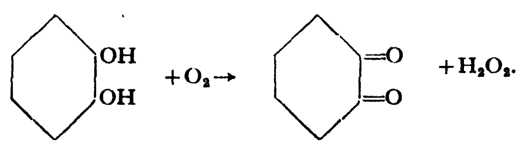


Peroxide form

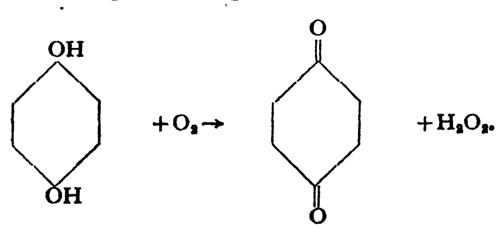
However, Szent-Györgyi gave no proof of the actual identity of the diketoquinone.

The now widely held view that orthodiketoquinone is formed by the enzymic oxidation of catechol has received support from the work of Pugh & Raper (1927), who isolated the crystalline anilino-orthoquinone compound formed by the action of mealworm tyrosinase (an enzyme very similar to catechol oxidase) on catechol in the presence of aniline. They succeeded in isolating and identifying by synthesis the anilino-orthoquinones obtained from phenol, catechol, *p*-cresol, *m*-cresol and homocatechol. By manometric methods they showed that the amount of oxygen taken up in the oxidation of catechol is greater than the equation for orthoquinone formation would show, and they considered that the orthoquinone took part in some further oxidation reaction of which they did not attempt any explanation.

This last experimental observation was supported by the previous work of Robinson & McCance (1926), who showed that orthoquinone is not the end-product of the action of tyrosinase on *p*-cresol. By measuring the amount of oxygen required for the oxidation of a known quantity of *p*-cresol or catechol by tyrosinase, it was found that three atoms of oxygen per molecule of *p*-cresol and two atoms per molecule of catechol were required. Theoretically, for the formation of orthoquinone only two atoms and one atom respectively of oxygen are required unless hydrogen peroxide is formed and is not decomposed at once. If the reaction of catechol oxidase on catechol in the presence of oxygen be accepted as orthodiketoquinone formation and hydrogen peroxide be formed, then the reaction may be represented as follows:



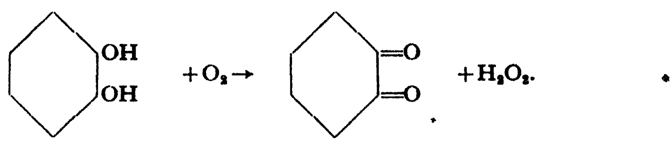
Onslow & Robinson (1926), using potato catechol oxidase, put forward evidence to show that hydrogen peroxide is formed in the enzymic oxidation of catechol, and this conclusion was supported by Platt & Wormall (1927). Wieland & Fischer (1926) obtained by fractional precipitation from *Lactarius vellereus* a preparation which oxidized hydroquinone to quinone with formation of the theoretical amount of hydrogen peroxide according to the equation:



Their catalyst was, however, essentially different from true catechol oxidase. It was thermostable and dialysable through fish bladder. Later experiments (1928) with purified thermolabile *Lactarius* enzyme resulted in no trace of hydrogen peroxide formation. Using this preparation which was completely free from catalase and peroxidase, a secondary consumption of any hydrogen peroxide formed

was out of the question. On mixing this latter preparation with the thermostable preparation, they found that the constant amount of hydrogen peroxide was formed. It would appear probable therefore that hydrogen peroxide is not a product of the oxidation of catechol but is probably formed by some system which is removed by the purification of the oxidase.

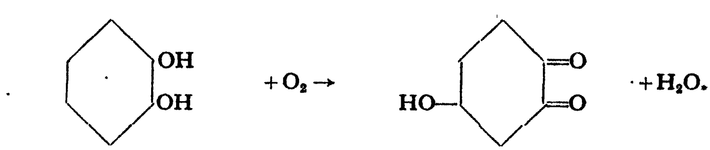
The most recent work on the problem of the enzymic oxidation of catechol, that of Wagreich & Nelson (1936), who used mushroom tyrosinase, throws doubt on the theory that orthoquinone is the final oxidation product. They found that the formation of the dianilino-orthoquinone, whether from orthoquinone and aniline or from a higher oxidation product and aniline, involves further oxidation than shown in the equation:



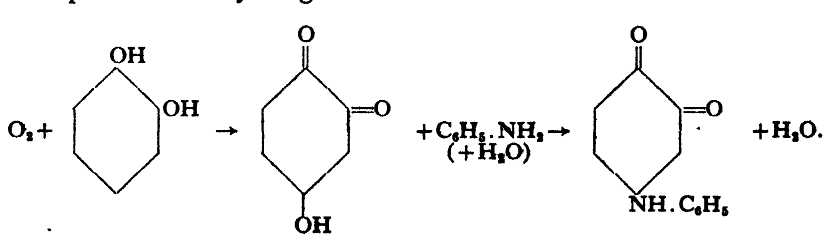
for three atoms of oxygen are used in the oxidation of one molecule of catechol in the presence of aniline, and the same dianilino-orthoquinone is obtained as described by Pugh & Raper (1927):

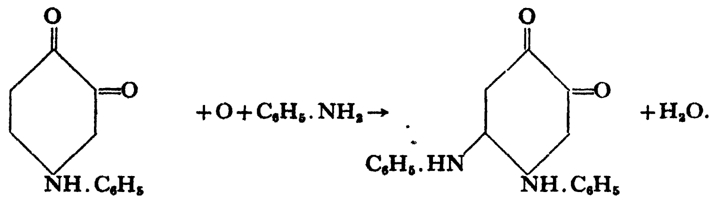
$$C_6H_4(OH)_2 + 2C_6H_5.NH_2 + 3O \rightarrow C_6H_2O_2(C_6H_5.NH)_2 + 3H_2O.$$

By adding aniline to the reaction mixture after catechol had previously been permitted to take up two atoms of oxygen per molecule of catechol and was therefore oxidized to a higher state than orthoquinone, the same dianilino-orthoquinone was obtained as when the catechol was oxidized in the presence of aniline. On measuring the amount of oxygen required to form the dianilino-compound when the catechol had previously taken up two atoms of oxygen per molecule, it was found to correspond to only one atom per molecule of catechol. Wagreich & Nelson suggest that the oxidation product of catechol is hydroxy-orthoquinone:



The formation of hydroxy-orthoquinone also agrees with the observation that only one additional atom of oxygen is taken up in the formation of dianilino-orthoquinone when aniline is added to the oxidized catechol. The following series of reactions is put forward by Wagreich & Nelson:





This theory has the advantage that in the oxidation of catechol the second atom of oxygen is accounted for and it is not necessary to suppose that hydrogen peroxide is formed. The production of hydrogen peroxide in the enzymic oxidation of catechol is still a matter of some doubt in spite of the work of Onslow & Robinson. It is, however, possible that different products are obtained by the action on catechol of oxidases from different sources, mushrooms and other fungi, higher plants and mealworms.

These observations on the oxidation products of catechol under the activity of catechol oxidase are the results of *in vitro* experiments. The nature of the oxidation product in the tissue may be different, for it has yet to be shown that in plant tissue catechol is the substrate oxidized. In the potato it is most certainly not the substrate, as catechol has never been found in the tubers. Most probably, judging by colour reactions, the substrate is a catechol tannin. In addition, one of the most widespread of Palladin's "respiratory chromogens", chlorogenic acid, is a natural tannin. The oxidation of these naturally occurring catechol compounds has yet to be investigated, and we know nothing concerning the nature of the oxidation products or the formation of hydrogen peroxide from catechol tannins.

Attempts have been made to ascertain whether catechol oxidase itself can act as a dehydrogenase activating the hydrogen of the phenol and transporting it to a hydrogen acceptor other than atmospheric oxygen. Onslow & Robinson (1926) attempted to oxidize catechol anaerobically with potato catechol oxidase, molecular oxygen being replaced by other hydrogen acceptors such as methylene blue, but without success. McCance (1925) believed that in the case of tyrosinase from *Lactarius vellereus* and mealworms, oxygen could be replaced by methylene blue as hydrogen acceptor, but the experiments of Pugh (1934) disproved McCance's conclusions. Catechol oxidase appears to be quite specific to oxygen as acceptor, and in addition therefore to a pronounced substrate specificity shows also a great acceptor specificity.

The distribution of catechol oxidase

Onslow (1921 *a*, *b*) examined 300 species in 293 genera and 173 natural orders of angiosperms and obtained a positive reaction for the presence of catechol oxidase, using the guaiacol test, in about 60 % of the cases examined. Of 64 % of the monocotyledonous orders examined 79 % gave a positive reaction, and of 60 % of the dicotyledonous orders examined 60 % gave a positive reaction. A higher percentage of the Sympetalae contained the system than of the Archichlamydeae. Szent-Györgyi (1927) examined a number of tissues, namely, potato, apple, pear, banana and the hull of the bean (*Phaseolus vulgaris*), which give a positive guaiacol reaction

throughout their tissues; *Beta vulgaris*, grape, carrot and celery which only give a positive reaction in parts; *Cucumis melo*, *Ananas sativa*, *Raphanus sativus niger* and *Cucumis sativus*, which give a negative test throughout. His conclusion was that in all those plants which give a negative guaiacol reaction, the tissues did actually contain a phenol oxidase system, and that the failure to give a positive reaction was due to one of two causes. Either the tissue did not contain a catechol derivative and therefore the orthoquinone which actually oxidizes the guaiacol to give the blue colour could not be formed, or the tissue contained a phenol oxidase oxidizing a phenol to a compound which is not an orthoquinone (but may be, for example, a monoquinone) and which is not capable of oxidizing guaiacol. In the former case the addition of catechol results in the blueing of guaiacol, while in the latter the catechol oxidase appears incapable of oxidizing catechol to an orthoquinone and therefore even in the presence of this substrate a blue colour is not obtained with guaiacol. Each group, according to Szent-Györgyi, contains some kind of catechol oxidase system, and he concluded that this enzyme system is much more widespread than the results of Onslow indicated.

The place of the catechol oxidase system in cell respiration

Palladin (1908) considered that plant oxidases do not act directly on the substance which is irreversibly oxidized but rather on phenolic substances in the tissue, converting them into quinones which have a higher oxidizing power than the original phenol. In modern terms the phenol is oxidized to a quinone, atmospheric oxygen being the hydrogen acceptor. The quinone acts as a hydrogen acceptor receiving hydrogen from some oxidizable substrate and returning to the phenol. This latter reaction may or may not be enzymic. The phenol acts therefore as a hydrogen transporter. The catechol oxidase system involves an orthodihydroxy compound as the hydrogen transporter, and usually the orthoquinone as the hydrogen acceptor which may involve a dehydrase. The hydrogen transporter may not necessarily be an orthodihydroxy compound, as the following experiment of Szent-Györgyi (1927) seems to indicate. He obtained from the radish by alcohol extraction and treatment with lead acetate, a substance which after decomposition with sulphuric acid yielded a solution which on addition of catechol oxidase gave a brownish yellow colour on standing. This coloration he attributed to the presence of some aromatic hydrogen transporter other than an orthodihydroxy compound.

According to this theory the following cycle of reactions occurs in tissues:

(1) Reduced H transporter + $O_2 \xrightarrow[\text{oxidase}]{\text{catechol}}$ H acceptor + H_2O or H_2O_2.

(2) H acceptor + oxidizable substrate (H donator) $\xrightarrow{\text{dehydrase}}$ reduced H transporter + oxidation product(s) of substrate.

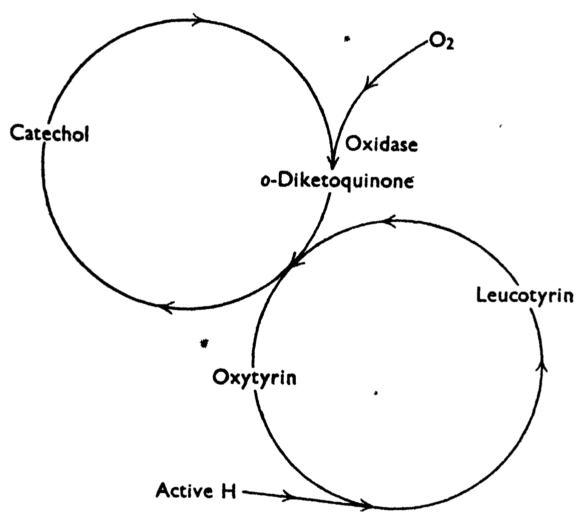
In the special case of Szent-Györgyi's first group of tissues, the series of reactions occurring may be represented:

(1) Orthodihydroxy compound $+ O_2 \xrightarrow[\text{oxidase}]{\text{catechol}}$ orthoquinone $+ H_2O_2$.

(2) Orthoquinone + oxidizable substrate $\xrightarrow{\text{dehydrase}}$ orthodihydroxy compound + oxidation product(s) of substrate.

It is probable that the hydrogen peroxide formed is either broken down to water and oxygen by the action of catalase which appears to be universal in its distribution, or it may take part in oxidation processes with peroxidase, which is present in almost all the higher plants. Which of these alternatives actually occurs may depend upon the concentration of hydrogen peroxide formed in the tissues, for catalase only decomposes hydrogen peroxide when the latter is at a much higher concentration than peroxidase requires (Meldrum, 1934). It is difficult to detect experimentally which reaction does occur, for hydrogen peroxide has only once been observed in the free state in tissues, by Callow (1923), who detected hydrogen peroxide in certain bacteria after their transference from anaerobic into aerobic conditions. The fact that hydrogen peroxide has only once been found in the free state is not evidence against the theory that it is formed; indeed, the more reactive the substance, the less likely is it to be found in the free state.

The position of this catechol oxidase system in the respiratory mechanism in plant tissues has been a matter of some dispute. It appears probable that if this system is concerned with the introduction of oxygen into the respiratory mechanism, it is not universal in plant tissues. Szent-Györgyi (1925) put forward a scheme of oxidations in the potato tuber in which catechol, catechol oxidase and a substance which he claimed to have isolated and named "tyrin" took part. This scheme was as follows:



Thus a cyclic scheme is produced in which the substrate which provides the active hydrogen is oxidized. But Platt & Wormall (1927) showed that the reactions attributed to "tyrin" by Szent-Györgyi could be accounted for by the presence of certain nitrogenous compounds. "Tyrin" appears to contain among other sub-

stances a mixture of amino-acids, and as all the properties attributed to it can be ascribed to the latter, it is considered that "tyrin" plays no role as a respiratory pigment.

Szent-Györgyi & Vietorisz (1931) concluded from their experiments on the rates of oxygen uptake of minced and whole potato tuber tissue that there are present in potato tubers an enzyme and its substrate, an orthodihydroxy compound, which, in the uninjured cells, are kept separate from one another and that the system only operates when the cells are mechanically injured. These workers concluded that, if this system plays any part at all in normal cell respiration, only a very small part of its activity is utilized. They consider that the system has quite another significance, namely, that it acts as a means of producing natural immunity when the cells have been injured by bacteria or otherwise. Quinones are known to have a strong bactericidal action, and Florkin (unpublished) claims that the quinone kills injurious micro-organisms and that the action of the quinone on proteins stops the entry of the invaders. Szent-Györgyi & Vietorisz (1931) were the first to suggest that the quinone formed on injury acts with, or accelerates the formation from albuminoid matter of an impervious layer which forms a mechanical protection to the injured surface.

Sutter (1936), however, considers that the increased oxygen uptake of minced tissues depends upon the facilitated diffusion of oxygen. In the intact tissue oxygen diffuses slowly, so that the rate of orthoquinone formation does not exceed the rate of its reduction by the cell substrate, and thus the orthoquinone does not accumulate. In damaged cells, the oxygen has unhindered access, and the supply of cell substrate is eventually stopped also as a result of the injury, and the orthoquinone then accumulates. The essential significance of the catechol oxidase system according to Sutter lies in its role in plant respiration.

Boswell & Whiting (1938) have put forward evidence showing that the catechol oxidase system does play a part in the respiration of isolated slices of potato tuber consisting of uninjured cells. It was shown that at least two-thirds of the respiratory activity of potato tuber slices was due to the action of the catechol oxidase system. The remaining respiratory activity which seems to vary from 10 to 33 % of the total activity according to season, is due to some system other than the catechol oxidase system. The respiratory quotient of both systems is unity. The naturally occurring orthodihydroxy compound of the potato tuber appears to be alternately oxidized and reduced, in the first stage being responsible for the oxygen uptake, and in the second stage taking part in a reaction with a hydrogen donator, this reaction probably being catalysed by a dehydrase. This latter reaction proceeds with or is immediately followed by evolution of carbon dioxide from the oxidized hydrogen donator. The orthodihydroxy compound and the catechol oxidase system are therefore not only responsible for two-thirds at least of the oxygen uptake of the tissue, but are also intimately connected with the output of carbon dioxide of the tissue.

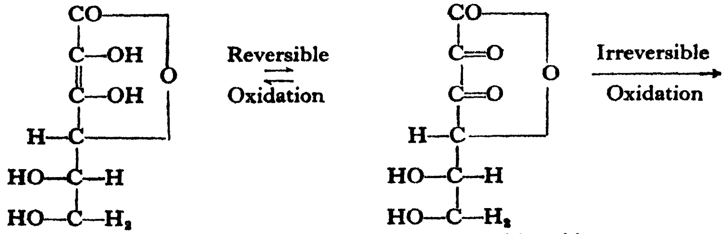
The technique was extended to other tissues, and it has been shown that a considerable part of the respiratory activity of slices of carrot root and artichoke tuber is under the control of the catechol oxidase system.

There is therefore evidence from those tissues which contain catechol oxidase and its associated orthodihydroxy compound that the system has an important part in the respiratory mechanism, in that it is closely connected with the oxygen uptake and carbon dioxide output. While this conclusion is the result of work done using isolated slices, it is not unreasonable to assume that the system may also be concerned in the respiratory activity of whole tubers. The difference in intensity of respiration (carbon dioxide output and oxygen uptake per unit weight per unit time) between whole tissues and isolated slices is more probably due to the greater availability of oxygen under the latter condition, than to a fundamental difference in respiratory mechanism. When potato tubers are used, increased sugar content as a wound reaction may also be a factor.

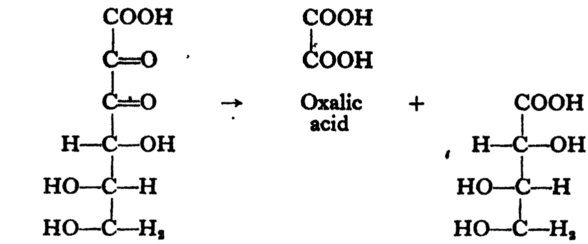
(2) *Ascorbic acid oxidase system*

The second of these oxidase systems to be discovered was the ascorbic acid oxidase system. Szent-Györgyi (1928) observed that the guaiacol test for peroxidase in turnip juice was only given after the addition of a large amount of hydrogen peroxide, and he considered that this was due to the fact that much of the hydrogen peroxide was reduced by some active "reducing factor", which showed reversible oxidation, present in the juice. This reducing factor, which Szent-Györgyi named hexuronic acid, was obtained from many plant tissues, and Svirbely & Szent-Györgyi (1932) showed that hexuronic acid was identical with vitamin C or *l*-ascorbic acid.

Szent-Györgyi (1931) found that hexuronic acid was present in cabbage leaves in the reduced state but disappeared on mincing owing to oxidation. The pulp did not reduce the oxidized hexuronic acid. Therefore the mechanism which brings about reduction of oxidized hexuronic acid is damaged by mincing to a greater extent than is the oxidation mechanism. The oxidation was shown to be due to an enzyme, hexoxidase, which was present in the juice. Hexoxidase or ascorbic acid oxidase is only slightly sensitive to cyanide and does not catalyse the oxidation of catechol, pyrogallol, quinol, *p*-phenylenediamine or leuxoindophenol. Ascorbic acid oxidase oxidizes *l*-ascorbic acid and its analogues, also reductic acid and reductone specifically as shown by Snow & Zilva (1938). Szent-Györgyi (1930) thought that the oxidation of ascorbic acid catalysed by ascorbic acid oxidase was a complex reaction and that less than 25 % of the substrate was oxidized. However, Tauber *et al.* (1935), using ascorbic acid oxidase isolated from *Cucurbita maxima*, showed that the enzymic oxidation of ascorbic acid was a simple linear reaction. The product of the first reversible stage of oxidation is dehydroascorbic acid, but if this is oxidized further irreversible products are formed:



l-Ascorbic acid Dehydroascorbic acid



Diketogulonic acid l-Threonic acid

The enzymic oxidation of certain analogues of *l*-ascorbic acid (*d*-arabo-ascorbic acid, *l*-gluco-ascorbic acid and *l*-galacto-ascorbic acid) also proceeds at a linear rate, but the rate of dehydrogenation of other analogues (*d*-ascorbic acid, *l*-arabo-ascorbic acid, *d*-gluco-ascorbic acid and *d*-galacto-ascorbic acid) falls with time (Johnson & Zilva, 1937*b*).

Autoxidation of *l*-ascorbic acid occurs in the presence of traces of copper or iron above *p*H 1·07 (Barron *et al.* 1936), but below *p*H 4·5 oxidation only proceeds to the reversible product. Above *p*H 4·5 the *l*-ascorbic acid which can be recovered from the oxidation product decreases until at *p*H 7·6 only 4 % of the oxidation product can be recovered as *l*-ascorbic acid. This *p*H effect has also been observed in the enzymic oxidation of *l*-ascorbic acid (Borsook *et al.* 1936).

Irreversible oxidation of ascorbic acid is prevented in plant tissues either by the presence of some strong reducing mechanism such as glutathione, or by the very low *p*H of the tissue. Mawson (1935) showed that lemon juice contained no protective mechanism other than the low *p*H which is less than 3·6. Hopkins & Morgan (1936) showed that glutathione protects *l*-ascorbic acid from oxidation in the presence of ascorbic acid oxidase. Only when the glutathione has practically disappeared can ascorbic acid oxidation be observed. Two molecules of glutathione are required for the protection of one molecule of ascorbic acid. In living tissues the two hydrogen atoms which in effect are transferred from each activated ascorbic acid molecule to oxygen are replaced by hydrogen from two molecules of glutathione. Glutathione also protects ascorbic acid from copper catalysed oxidation, but here the protective mechanism does not depend upon hydrogen transference but upon direct inhibition as glutathione forms a stable compound with copper. Crook & Hopkins (1938) examined the *p*H effect of protection by glutathione and found that at *p*H 6 there was complete protection of ascorbic acid by glutathione. In addition they found that in cabbage, cauliflower and to a less extent in cucumber juice there was a mechanism which catalysed the reduction.

Ascorbic acid oxidation is catalysed not only by ascorbic acid oxidase but also indirectly by peroxidase and phenolases. Szent-Györgyi (1928) showed that pure peroxidase and hydrogen peroxide had no effect on ascorbic acid, but only oxidized it in the presence of juice of *Brassica oleracea*. He thought that a phenolic body might be the intermediate catalyst. Johnson & Zilva (1937*a*) showed that crude apple or potato juice catalysed ascorbic acid oxidation. This was considered to be due to the indirect action of a phenolase and a phenolic body in the juice. There are therefore three enzymes which are capable of catalysing ascorbic acid oxidation,

peroxidase and phenolases which act indirectly, and ascorbic acid oxidase which acts directly. Ascorbic acid oxidase has been demonstrated in a number of tissues, namely, cabbage (Szent-Györgyi, 1930), *Cucurbita maxima* (Tauber *et al.* 1935), cauliflower (Hopkins & Morgan, 1936), pods of *Moringa pterygosperma* (Srinavasan, 1936), lettuce, peach and watercress (Barron *et al.* 1936), pumpkin, pea, string bean, lima bean, sweet corn, Swiss chard, carrot, parsnip and spinach (Kertesz *et al.* 1936), cucumber and marrow (Johnson & Zilva, 1937*a*). Ascorbic acid oxidase does not appear to be as widespread among plant tissues as other oxidases. Banga & Philippot (1939) examined twenty-five plants, and of these only four showed the presence of ascorbic acid oxidase.

Ascorbic acid is much more widespread among plant tissues than ascorbic acid oxidase. In addition to the tissues containing ascorbic acid oxidase, ascorbic acid is present in many other tissues such as apple and potato. Bracewell *et al.* (1931) showed that in the apple the peel is richer in ascorbic acid than the inner part. Paech (1938) has shown that the inner part of the potato is richer in ascorbic acid than the outer part. Lunde & Lie (1938) found that vitamin C is widespread among the brown and red algae.

There is no experimental evidence that ascorbic acid acts in tissue respiration in a similar manner to orthodihydroxy compounds in catechol oxidase tissues. Indeed, it seems more probable that ascorbic acid plays no part at all in the introduction of oxygen into the respiratory system. Glutathione which is thought to be present in all tissues is immediately oxidized by dehydro-ascorbic acid with the formation of ascorbic acid. Since there is good evidence that glutathione is oxidized by a different mechanism, if the ascorbic acid system is concerned with introduction of oxygen, it is only as a subsidiary to the glutathione system which itself is now considered unimportant in respiration. Zilva *et al.* (1938) showed that *l*-ascorbic acid was present in both reduced and reversibly oxidized forms in the apple, and although the total quantity of *l*-ascorbic acid present remained constant per unit weight throughout the growth of the apple, there was a change in the relative proportions of the two forms, for as the fruit approached maturity the proportion of the reduced form increased and that of the dehydro-ascorbic acid decreased. These workers consider that in the apple ascorbic acid acts as a carrier between the catechol oxidase system and some other system. The change in the equilibrium of the two forms of *l*-ascorbic acid they consider a function of some metabolic process connected with the growth of the apple. Pett (1936) found that the contents of ascorbic acid and glutathione in stored potato tubers fell steadily and simultaneously during storage, but were restored, also simultaneously on the commencement of sprouting. Owing to the small number of plants which contain the complete ascorbic acid oxidase-ascorbic acid system, it is probable that the importance of this system does not lie in the introduction of oxygen into the respiratory mechanism. The more widespread distribution of ascorbic acid alone suggests that it may act as a hydrogen transporter in connexion with another complete oxidase system. In more general terms, it may control to some extent the delicate equilibrium of the complex reactions proceeding simultaneously in cell oxidations.

(3) *Dihydroxymaleic acid oxidase system*

The dihydroxymaleic acid oxidase system was discovered during work on the oxidation mechanisms of catechol oxidase and ascorbic acid oxidase. Szent-Györgyi (1934) found that on addition of a ferrous salt to a neutral solution of catechol or ascorbic acid, a violet colour was produced. This colour reaction was given with equally strong intensity by only three of many substances examined (Szent-Györgyi, 1938), namely, catechol, ascorbic acid and dihydroxymaleic acid, although a few substances, cystein, oxaloacetic acid, salicylic acid and acetyl-acetone, showed a slight reaction. It was noticed that catechol and ascorbic acid were the central members of two plant oxidation systems, while dihydroxymaleic acid is an oxide of succinic acid which itself is the central member of the cytochrome oxidase-succinic acid oxidation system of animal tissues. Banga & Szent-Györgyi (1938) thought that dihydroxymaleic acid might play an analogous role in a plant oxidation system.

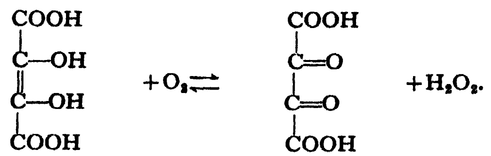
It has already been shown that many plant tissues contain a catechol oxidase system, some contain an ascorbic acid oxidase system, and a few contain both systems, but in many plants neither of these systems appears. In these latter plants Banga & Szent-Györgyi (1938) considered that there might be a dihydroxymaleic acid oxidase system analogous with the other two systems. These workers were able to show the occurrence in horse-radish root juice of an enzyme which catalysed the oxidation of dihydroxymaleic acid in air, and found that one atom of oxygen was used for each molecule of acid oxidized:

$$\begin{array}{l} \mathrm{COOH} \\ | \\ \mathrm{C{-}OH} \\ \| \\ \mathrm{C{-}OH} \\ | \\ \mathrm{COOH} \end{array} + \mathrm{O} \xrightarrow[\text{Oxidase}]{\text{Dihydroxymaleic acid}} \begin{array}{l} \mathrm{COOH} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{C{=}O} \\ | \\ \mathrm{COOH} \end{array} + \mathrm{H_2O}.$$

The dihydroxymaleic acid oxidase was obtained from horse-radish root juice by addition of excess acetone or alcohol to the juice previously cleared by freezing, thawing and leaving to precipitate. The enzyme showed an optimum *p*H between 3 and 6, did not catalyse the oxidation of catechol or ascorbic acid, and appeared to be specific for the oxidation of dihydroxymaleic acid. A number of tissues were tested for the presence of dihydroxymaleic acid oxidase, and its presence was demonstrated either alone or together with catechol oxidase, ascorbic acid oxidase or both, in a number of tissues. Asparagus was found to contain a very active dihydroxymaleic acid oxidase, but neither catechol oxidase nor ascorbic acid oxidase, while cucumber was found to contain a powerful ascorbic acid oxidase and some dihydroxymaleic acid oxidase but no catechol oxidase; rape root contained an active dihydroxymaleic acid oxidase and a less active ascorbic acid oxidase, while in the leaves the content was inverted, and neither organ contained catechol oxidase.

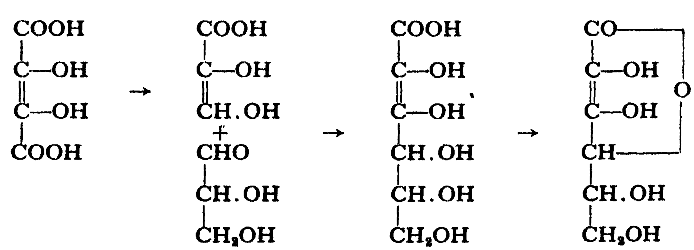
Banga *et al.* (1938) obtained by acetone precipitation from the leaves of *Rumex acetosa* an active dihydroxymaleic acid oxidase preparation and found that the enzyme was completely inhibited by 0·0001 M cyanide at *p*H 4, that is at its optimum

*p*H. They showed that the substrate was oxidized reversibly the hydrogen being oxidized to hydrogen peroxide:



They concluded that dihydroxymaleic acid oxidase was one of the three basic aerobic oxidases of plant tissues, the other two being catechol oxidase and ascorbic acid oxidase.

Banga and Philippot (1939) claim to have shown the presence of a dihydroxymaleic acid oxidase in potato tubers. Unpublished results of the authors on the effect of dihydroxymaleic acid on the respiration of potato slices show, however, that this compound does not act in the respiratory mechanism in a similar way to the naturally occurring orthodihydroxy compound. The dihydroxymaelic acid does not form part of a direct oxidase system in the potato tuber. It appears to be oxidised by hydrogen peroxide in the presence of peroxidase. There is as yet no evidence as to whether or not it forms a cyclic system being alternately oxidised and reduced. Carbon dioxide is liberated during the oxidation by a reaction which involves a dehydrogenase. Dihydroxymaleic acid has not yet been demonstrated with certainty in plant tissues, but detection of this substance is very difficult and it cannot be expected in large quantity since it is very labile in the free state. The small quantity necessary for the dihydroxymaleic acid oxidase mechanism is probably stabilized by adsorption. Dihydroxymaleic acid is nearly related to common plant acids such as malic, succinic and citric acids, and it can be formed from them by oxidation. It is possible that ascorbic acid is formed from dihydroxymaleic acid by one-sided decarboxylation followed by condensation with glyceraldehyde:



Banga & Philippot (1939) investigated twenty-five plants, including celery, beetroot, dock, onion, lettuce, cress, parsley, cucumber, radish, spinach, potato and carrot, and found that all of them contained dihydroxymaleic acid oxidase, thirteen contained a phenolase and only four contained ascorbic acid oxidase. The activity of the dihydroxymaleic acid oxidase in most of the tissues examined was great, as 0·1 ml. of juice in many cases oxidized 5 mg. dihydroxymaleic acid in 5 min. The dihydroxymaleic acid oxidase preparations from different sources show some variation in properties in the same way as preparations of catechol oxidase

from different sources. Two types of enzyme may be distinguished in regard to the *p*H effect, namely, the dock type and the cress type. The dock type of enzyme shows a sharp optimum activity at *p*H 4·1, while the cress type shows with rising *p*H an increasing activity. The dock type is found in dock, potato and spinach, while the cress type is found in cress, cucumber, cauliflower and cabbage. Three types of dihydroxymaleic acid oxidase are observed when the effect of 0·01–0·001*M* cyanide is studied. The enzyme from many tissues, including dock, radish, cucumber and spinach, shows total inhibition, that from cress, cabbage, carrot and lettuce is partially inhibited, while that from cauliflower and potato is activated. It seems highly probable that dihydroxymaleic acid oxidase is a metal proteid. Szent-Györgyi (1938) has shown that the entry of cyanide into a metal complex may either inhibit or promote the catalytic action of the metal.

A mechanism of dihydroxymaleic acid oxidation has been proposed by Banga & Philippot (1939). They found that in experiments, *in vitro*, the hydrogen peroxide formed in the oxidation of one molecule of dihydroxymaleic acid was used for the oxidation of a second molecule of dihydroxymaleic acid. Hydrogen peroxide alone, however, only oxidizes the acid very slowly even in the presence of peroxidase. In the plant tissues investigated dihydroxymaleic acid oxidase was always found accompanied by a very active peroxidase and a polyphenol. It was concluded that the phenol was oxidized to a quinone by the action of hydrogen peroxide and peroxidase, then the quinone formed oxidized a second molecule of dihydroxymaleic acid. When the polyphenol was removed from the plant juice, dihydroxymaleic acid was oxidized at approximately half the rate observed in the presence of the polyphenol. The addition of catechol or boiled juice restored the original velocity of oxidation. The authors have observed that the addition of dihydroxymaleic acid to the washed slices of potato tubers results in a large increase in oxygen uptake. This increase is not permanent and the rate of oxygen uptake falls finally to the pre-addition value. Experiments involving the use of specific inhibitors lead to the conclusion that the oxidation of dihydroxymaleic acid does not involve either a direct oxidase system or a dehydrogenase. This demonstration of the absence of a dehydrogenase invalidates the theory of Banga and Philippot. It appears that the indirect oxidase system—hydrogen peroxide and peroxidase—is responsible in potato tubers for the oxidation of dihydroxymaleic acid. This is in agreement with the conclusions of Swedin and Theorell (1940). They examined a number of species including *Rumex acetosa* and concluded that there was no sufficient reason for the assumption that dihydroxymaleic acid oxidase and peroxidase are different ferments. *R. acetosa* was one of the species which Banga *et al.* (1938) regarded as containing the dihydroxymaleic acid oxidase system.

It would appear therefore that the existence of a dihydroxymaleic acid oxidase is uncertain and that further observations are required before the correct position can be established.

III. Other systems

Alloxazin derivatives

Alloxazin and its derivatives can be reversibly oxidized and reduced, the oxidation being either through the reduction of some other compound or by molecular oxygen. The demonstration that the derivative, alloxazin-adenine-dinucleotide was the coenzyme or prosthetic group of a number of oxidation catalysts present in animal tissues, including *d*-amino acid oxidase, xanthine oxidase and Warburg's yellow ferment raised it to an important place in the respiration system of animal tissues. It is also probable that alloxazin derivatives are the prosthetic groups of other as yet unidentified enzymes.

The observations of Kuhn *et al.* (1934) that lactoflavin (a derivative of alloxazin) is present in apple juice, bananas, apricots, tomatoes, hips, haws, malt extract, carrots, spinach (fresh and dried), dried lucerne, fresh grass and wheat bran raises the question as to the part which alloxazin derivatives may play in plant respiration, possibly as the prosthetic groups in oxidizing enzymes.

Iron-porphyrin compounds

These are a most reactive group, and by virtue of their capacity to combine with nitrogenous compounds to give complexes with a wide range of properties play most important parts in all phyla of the biological kingdom, both plant and animal. Their part in the oxidation-reduction systems of higher plants is twofold, alone or combined with proteins they form the three cytochromes *a*, *b* and *c*, and associated with another protein they form peroxidase.

Cytochrome

Of all iron prophyrins cytochrome is one of the most important. Keilin (1925) reported its presence in the higher plants. Recently, Hill & Bhagvat (1939) have observed its presence in the cotyledons of the germinating seeds of *Phaseolus*. Our knowledge of cytochrome is chiefly due to the work of Keilin, using material isolated from yeast and animal tissues. He divided cytochrome into three fractions, cytochromes *a*, *b* and *c*. The exact constitution of the first two is unknown and they are reported as being autoxidizable. Cytochrome *c* is not autoxidizable, and according to Theorell (1938) it is an iron porphyrin unit linked to two sulphur-containing side chains which through peptide linkages join to a protein molecule. Cytochrome *c* is only oxidized in the presence of cytochrome oxidase which Keilin considers to be a metal-protein compound. There is as yet little information available concerning the distribution of cytochrome among the higher plants or of the part which it plays in respiration. Hill & Bhagvat report that the cytochrome system in the cotyledons of germinating *Phaseolus* seeds is associated with a succinic dehydrase, which brings the respiratory mechanism of these seeds into line with that found in yeast and animal tissues.

Peroxidase

Peroxidase appears to be an iron-porphyrin protein (Kuhn, *et al.* 1931; Keilin & Mann, 1938), which catalyses oxidations in the presence of hydrogen peroxide (Wieland & Sutter, 1928). Peroxidase would appear to play an intermediate role in respiration, utilizing the peroxide formed in the activity of other oxidation systems, e.g. polyphenol oxidase. Hydrogen peroxide may be formed in each of the three oxidase systems, polyphenol oxidase, ascorbic acid oxidase, and dihydroxymaleic acid oxidase. It is therefore possible that peroxidase acts with the hydrogen peroxide formed in each system to bring about the oxidation of more substrate. For example, in the polyphenol oxidase system the polyphenol is oxidized with the probable formation of hydrogen peroxide, and that hydrogen peroxide may bring about the oxidation of more polyphenol.

O_2 + polyphenol oxidase + polyphenol → quinone
↓
Peroxidase + H_2O_2 + polyphenol ↗

A similar reaction series occurs according to Banga & Philippot (1939) in the dihydroxymaleic acid system.

O_2 + dihydroxymaleic acid oxidase + dihydroxymaleic acid → diketone
↓
Peroxidase + H_2O_2 + dihydroxymaleic acid ↗

In the case of ascorbic acid oxidase however, hydrogen peroxide and peroxidase cannot oxidize ascorbic acid directly, and here Szent-Györgyi (1938) considers that the hydrogen peroxide formed together with the peroxidase oxidizes phenylbenzopyran (flavone) which acts as a hydrogen transporter and oxidizes the ascorbic acid:

O_2 + ascorbic acid oxidase + ascorbic acid → dehydroascorbic acid
↓
Peroxidase + H_2O_2 + flavone
↓
Oxidized flavone + ascorbic acid ↗

Elliott (1932) tested the effect of peroxidase and hydrogen peroxide on many compounds, and the following were not oxidized: formate, oleate, acetate, lactate, glucose, fructose, glycerol, ethyl alcohol, acetaldehyde, glycine, glutamic acid, phenylalanine, histidine, brucine, nitrite and tryptophase. Formaldehyde, dihydroxyacetone and phenylglyoxal were oxidized by very dilute hydrogen peroxide without peroxidase. Only phenolic compounds were oxidized by peroxidase and hydrogen peroxide.

Hydroxynaphthoquinones

A number of substances have been isolated from plant tissues which are derivatives of hydroxynaphthoquinones and which form reversible oxidation-reduction systems.

Fieser (1927) gives a list of woods from which one derivative, lapachol, has been isolated. Lomatiol has been isolated from the seeds of two species of *Lomatia*, *L. ilicifolia* and *L. longifolia*. Friedheim (1934) reported on the potentials of two derivatives, Lawsone from the henna plant, *Lawsonia inermis*, and Juglone from the husks of the walnut. Plumbagin, which is present in the roots and stems of *Plumbago europea*, was considered by Madinaveita & Gallengo (1928) to be a methyl juglone. Rennie (1893) reported the isolation of two pigments from the reddish sap in the tubers of *Drosera whittakeri*, which he believed to be trihydroxy- and dihydroxymethylnaphthoquinones.

The following table gives the E_0' values for some of these compounds as recorded by Ball (1934):

Table 1

Redox system	Potential, V.	Medium
Lapachol	+0·287	50 % alc.
Lomatiol	+0·294	50 % alc.
Lawsone	+0·352	Water
	+0·356	50 % alc.
Juglone	+0·435	Water
	+0·452	50 % alc.

Respiratory chromogens

Palladin (1912) described a number of substances isolated from plant tissues which on oxidation in air in the presence of an oxidase gave rise to pigments. These pigments were capable of oxidizing different organic substances—amino-acids and carbohydrates—being themselves reduced to the original colourless chromogens. This latter oxidation-reduction is carried out in the presence of an oxido-reductase. These chromogens and pigments may be the prosthetic groups loosely attached to certain unknown proteins, the whole forming an oxidase enzyme.

Oparin (1927) has studied chlorogenic acid as a typical respiratory chromogen. This compound is, however, a catechol derivative, and it is doubtful how far it is typical of the group of respiratory chromogens and if it should not rather be considered as the coenzyme of a metal-protein polyphenol oxidase. *In vitro* chlorogenic acid oxidized at *p*H 7·6 by a phenolase from germinating sunflower seeds is capable of oxidizing glycine with the production of carbon dioxide and ammonia. Cannan (1926) reported on the potentials shown by Hermidon under various conditions. This was a respiratory pigment isolated by Haas & Hill (1925) from *Mercurialis perennis*.

IV. Conclusion

The cellular respiration of carbohydrates in the higher plants is carried out through a series of oxidation-reduction systems which transport hydrogen from the initial substrate to molecular oxygen. The oxidase enzymes active in this series of oxidation-reduction systems of graded and increasing potential are as yet only very imperfectly recognized. The polyphenol oxidase system alone has been studied in great detail. Of the others, their place in the respiration chain is uncertain, for

while our knowledge of their activity in the animal kingdom may be great (cytochrome, alloxazin derivatives) we have only recently found that they are present in the higher plants. Finally, there are a number of compounds with a limited range of distribution in the plant kingdom which possess reversible oxidation-reduction properties but of their place in the respiration chain we know nothing.

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THE FORMATION OF PYRUVIC ACID IN BARLEY RESPIRATION

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It was shown in an earlier paper (James & Norval, 1938) that the enzyme carboxylase is present in barley and that pyruvic acid supplied to living barley leaves and embryos is broken down, increasing their CO_2 output and raising their R.Q. towards 1·2. No pyruvic acid normally accumulates in the tissues, since negative results were consistently obtained with the iodoform and ammonia-nitroprusside reactions. This was attributed to the activity of the carboxylase system. Addition of pyruvate reduced the rate of oxygen consumption and the rate of reduction of methylene blue under nitrogen; oxidation of pyruvic acid therefore seems unlikely. It was the aim of the present work to see if pyruvic acid is formed, as distinct from accumulated. The added fact of its formation while respiration was going on would make the evidence of its taking a part reasonably complete for our species. Respiration is here taken to include glycolysis, whatever the relation between glycolysis and oxidation may be.

Methods

The enzyme block. The general method was to poison the principal mechanism of pyruvate removal, i.e. carboxylase. This block should lead to accumulation of pyruvate in the tissues if it is being formed continuously, since oxidation appears to be ruled out. Preliminary experiments with bisulphite as a keto-fixative did not give promising results. Two types of carboxylase poison are available: (1) acetaldehyde, (2) the aromatic sulphonic acids. We have used acetaldehyde and three of the latter, viz.: 1-amino, 2-naphthol, 4-sulphonic acid; 1-naphthol, 2-sulphonic acid; and anthraquinone-2,7-disulphonic acid. The last gives a deep red solution, and the colour has been useful in proving its penetration into the tissues. In experiments with yeast carboxylase, Axmacher & Ludwig (1936) found 86% inhibition with 3×10^{-3} 1-naphthol-2-sulphonic acid and 77% inhibition with anthraquinone-2,7-disulphonic acid of the same concentration. We have made up concentrations of 3×10^{-3} and 2×10^{-3}, but the concentration within the tissues is uncertain.

Identification of pyruvic acid. The ammonia-nitroprusside reaction as already described (James & Norval) was used in preliminary experiments. This is not affected by even large doses of simple aldehydes or ketones such as acetaldehyde and acetone, but is given by oxaloacetic acid and perhaps by other keto-acids.

For isolation and more exact identification 0·5% 2,4-dinitrophenylhydrazine in $2N$ HCl was added to the prepared extract. The hydrazone formed was purified from hydrazine and non-acid hydrazones by repeated extractions from ethyl acetate first into saturated and then into $M/15$ sodium phosphate. The alkali was

acidified with $2N$ HCl and the hydrazone re-extracted into fresh ethyl acetate. The exchanges were repeated three or four times and the hydrazone precipitated by allowing the ethyl acetate to evaporate off at room temperature. The technique followed in all essentials the method of Peters & Thompson (1934). Rather elaborate methods of preparing the extract were found necessary and these are described under individual experiments, since they were varied.

A specimen pyruvic 2,4-dinitrophenylhydrazone was prepared by the above method from twice re-distilled pyruvic acid. On crystallizing slowly from ethyl acetate it gave small canary-yellow plates, mostly diamond-shaped, single and clustered. Its melting point was 216°. The hydrazone gave a deep red solution with alcoholic potash and a similar colour which was stable for several hours, with Lu's reagent (3 vol. 10% Na_2CO_3 + 2 vol. N NaOH in water). According to Lu (1939), colours given by some similar compounds fade rapidly. The hydrazone of α-ketoglutaric acid (M.P. = 222°), an acid likely to be present in plant extracts, gives a very poor colour in alcoholic potash, which disappears on addition of water.

When pyruvic acid 2,4-dinitrophenylhydrazone is thinly spread it volatilizes rapidly at about 190° C., and the conventional method of determining melting points cannot be used with small quantities. We found that satisfactory results could be obtained even with as little as two or three crystals, by using a simple hot-box method, as follows: The hydrazone was allowed to crystallize slowly from ethyl acetate on to a thin glass slip laid in the crystallizing dish. It was necessary to have crystals more than about 200 μ long if they were not to be entirely lost by volatilization. They were covered with a second slip and the glass-crystal-glass sandwich was then laid on a brass slab which was drilled with a vertical hole. A similar brass slab, grooved to accommodate the thickness of the slips, was then laid over and enclosing them. The crystals could be watched through a microscope adjusted over the vertical inspection-holes which passed through both brass slabs and was closed top and bottom with thick glass slides. A thermometer was placed in a horizontal bore drilled as a tangent to the vertical bore. The bulb itself was not in actual contact with the brass but touched the glass slips enclosing the crystals. Heat was applied to an extension of the lower brass slab on the side opposite the thermometer. The apparatus was calibrated with samples of the specimen hydrazone described above, giving M.P. = 216° by the conventional method. The apparatal correction was found to be + 5° C.

Experimental results

Roots (NH_3-*nitroprusside reaction*)

Before attempting an actual isolation, preliminary experiments were carried out with the NH_3-nitroprusside colour reaction. Barley grains (var. Plumage Archer) were soaked and germinated on muslin spread over small dishes of tap water. As the roots grew they penetrated the muslin and hung down into the water. After 3 days in a warm room, when the roots were ½ in. long or more, the tap water was removed and replaced by the poison. Samples of about twenty roots

were taken during the following days and pounded under 5 % trichloracetic acid in a mortar. The extract was decanted into a test-tube and tested for pyruvic acid.

The first series of experiments was carried out with a range of acetaldehyde concentrations from 0 to 0·1 %. The results are summarized below.

Table 1. NH_3-*nitroprusside reaction for pyruvic acid in barley roots*

Acetaldehyde %	Days						
	1	2	3	4	5	6	7
0 (tap water)	.	.	.	o	.	o	.
0·001	.	o	.	o	.	.	o
0·01	o	o	+	+	+	++	++
0·025	+	.	+	+	o	.	.
0·05	+	.	++	+	o	.	.
0·1	.	+	.	+	.	.	o

o = no reaction. + = weak reaction. ++ = moderate reaction.

The negative results without poisoning agreed with previous experience. With 0·01 % acetaldehyde, pyruvic acid was apparently slowly accumulated during the period observed. At the higher concentrations a positive reaction appeared more rapidly, but soon declined.

Similar experiments were performed with sodium salts of each of the three aromatic sulphonic acids mentioned above in 0·3 % concentration. Positive reactions were developed after 3 or 4 days, but were weaker than those obtained by using acetaldehyde. This was probably due to a much poorer penetration of the sulphonic acids into the root cells. The NH_3-nitroprusside reaction was quite insensitive to all the poisons at the concentrations employed.

These results suggest that an α-keto-acid, probably pyruvic acid, had been caused to accumulate in the roots. Since the accumulation was a direct result of carboxylase poisoning, it was unlikely that β- or γ-keto-acids (whose reactions with ammoniated nitro-prusside have not been studied) should be involved.

Leaves (2,4-dinitrophenylhydrazine method)

Although apparently more efficient as a carboxylase poison, acetaldehyde was considered objectionable as a possible source of pyruvic acid via oxidative resynthesis. We therefore employed 1-naphthol-2-sulphonic acid in our main experiments, with occasional recourse to anthraquinone-2,7-disulphonic acid. The roots, even at the absorbing zones, had proved rather slow in taking up these acids and were further found to have very slow respiration rates. Their formation of hydrazones was meagre. We therefore turned to a method which had earlier been found successful with pyruvic acid feeding (James & Norval, 1938) and with HCN poisoning (James & Hora, 1940). Leaves were cut from field plants (var. Plumage Archer) in the middle of the day in sunny weather and transferred to jars containing 0·2 % sulphonic acid solutions. They were left standing in a dark cellar for 16–24 hr. at a temperature of about 16° C. It was known that longer periods would have

involved a risk of carbohydrate starvation and formation of keto-acids from protein decomposition. The poison taken up in the transpiration stream comes into intimate contact with the leaf cells within about a quarter of an hour and is continuously supplied throughout the experiment.

At the end of the respiration period, the leaves were chopped up and pounded under 5% trichloracetic acid and hydrazones prepared from the extract by the method given. In every experiment in which a carboxylase poison was used, hydrazones, soluble in mild alkali, were obtained. In the first experiments of this kind the product was a mixed one. It was yellow-brown, crystallized poorly from ethyl acetate, or might even be an uncrystallizable oil. The melting point of the crystalline specimens was about 190–200° C. On addition of alcoholic potash all these preparations gave strong red colorations which were stable. Diamond-shaped crystals typical of pyruvate under the conditions of formation could sometimes be distinguished under the microscope. Additional exchanges between ethyl acetate and sodium phosphate did not improve matters, so it seemed clear that we had to do with the hydrazones of mixed keto-acids. Adsorption of the hydrazones from alcohol on to a column of calcium carbonate separated a red compound from a zone of canary yellow. The latter was redissolved in ethyl acetate and reprecipitated, but proved to be still a mixture. It was therefore necessary to attempt a purification of the extract before hydrazone formation. Some time was spent in discovering an effective way of doing this; the method finally adopted being the following:

A sample of about 100 young leaves was put to respire in the dark with their cut ends dipping into 0·2% 1-naphthol-2-sulphonic acid. After 22 hr. they were frozen overnight at about −12° C. The following morning they were thawed at room temperature and the sap pressed out at 2 tons/sq. in. The crude juice so obtained was precipitated with barium acetate adjusted to *p*H 8 with baryta. The barium precipitate was centrifuged off and the clear solution deproteinated by being raised to the boil with trichloracetic acid in a final concentration of 5%. The protein flocculum was filtered off and the clarified extract distilled in vacuo, water and other volatiles coming over at about 50° C. The distillate was treated with 2,4-dinitrophenylhydrazine and the hydrazone extracted as before. It now crystallized more satisfactorily. A zone of canary yellow crystals was deposited, though the last to form at the bottom of the dish were still reddish and were discarded. The pure yellow crystals were wide, flat plates with pointed ends, i.e. some angles of the diamonds were poorly developed, a condition also observed in the type preparation. A permanent port-wine red was given with alcoholic potash which was not discharged by adding water. This experiment was repeated with identical results. The melting point of the crystals obtained in the first experiment was above 208° C., at which temperature, being too small, they were lost by volatilization. The product of the second experiment was examined in the special hot-box (p. 267) and duplicate melting-point determinations agreed exactly with those for the preparation from twice redistilled pyruvic acid; M.P. = 216° C. (corrected). A pure pyruvic acid compound had therefore been isolated. A control sample of leaves receiving water instead of 1-naphthol-2-sulphonic acid yielded no hydrazones by this method.

Oxaloacetic acid, if present in an extract, slowly decarboxylates to pyruvic acid, even at room temperature, and a neutral *p*H. Formation of pyruvic acid in this way was guarded against in the final experiments by the immediate precipitation with barium at *p*H 8. This would remove oxaloacetic acid and other dicarboxylic acids.

SUMMARY

1. Barley roots poisoned with 0·01 → 0·1 % acetaldehyde or 0·3 % solutions of certain aromatic sulphonic acids developed an ammonia-nitroprusside reaction for pyruvic acid, which is not given by normal roots.

2. Pure pyruvic acid was isolated as the 2,4-dinitrophenylhydrazone from cut and darkened leaves poisoned with 0·2 % 1-naphthol-2-sulphonic acid.

Since these poisons inactivate carboxylase, the above results suggest that pyruvic acid is continuously formed by barley tissues and is normally decarboxylated. Further evidence is thus provided for regarding it as an intermediate product in barley respiration.

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A FORM OF LOW-TEMPERATURE INJURY IN DETACHED LEAVES

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(With 2 figures in the text)

At least two types of low-temperature injury are known to occur in plant tissues. At temperatures below the freezing-point of the cell sap changes occur which result in the death of the cells. There is a considerable literature on the mechanism of frost resistance in plants, and injury due to freezing has been extensively investigated. On the other hand, at temperatures above the freezing-point of the cell sap, various forms of low-temperature injury or "physiological breakdown" have been described. These changes do not necessarily cause the immediate death of the cells, though metabolism may be obviously affected in a number of ways.

Examples of forms of low-temperature injury belonging to this class have hitherto been recorded only for fruits. They include browning of the tissues in plums, "woolliness" in peaches, and the pitting of the skin in grapefruit. The "sweetening" of potatoes at low temperatures (Barker, 1933) may be a parallel phenomenon.

The kinetic aspects of cold injury in fruit have been examined by van der Plank & Davies (1937), who found in many cases temperatures of maximum injury, with "safe" regions above and below the critical region.

It is recognized that fruit exhibits considerable variation, individual and seasonal, in its susceptibility to low-temperature injury. It is generally held that unripe fruit is more susceptible than ripe fruit. Davies and his co-workers (1936) have shown that peaches may be protected against the development of woolliness by keeping the fruit for a few days at a high temperature before cooling. Peaches left on the tree till thoroughly ripe never develop woolliness in store. Donen (1939) has shown that there is an inverse relation between sorbitol content and low-temperature browning in the Kelsey plum.

As far as the writer is aware, low-temperature physiological breakdown has hitherto not been described in foliage leaves. In the course of experiments on the respiration of detached leaves of the loquat (*Eriobotrya japonica*), kept in the dark with their petioles in water, it was found that leaves picked in the winter and kept at temperatures in the neighbourhood of 2° C. became brown in patches when the temperature was subsequently raised. The browning was found to begin at the periphery of the leaf and between the main veins. It was accompanied by a sudden rise in CO_2 output followed by a fall. The cells in the affected areas eventually died.

Leaves kept at 25° C. for a few days before being placed in cold store developed a high degree of immunity to low-temperature injury. Low-temperature browning in leaves could therefore be controlled in exactly the same way as woolliness in peaches.

Attempts were made to discover whether similar phenomena could be demonstrated in leaves from other plants. It was found in most of the species investigated that the leaves would not stand up to the experimental treatment but wilted after a day or two. Of the leaves which did not wilt two evergreen types—*Viburnum* sp. and *Ficus* sp.—showed physiological breakdown after exposure to 2° C. None of the leaves from herbaceous plants showed any visible injury after storage at this temperature. These included *Tropaeolum majus*, *Helianthus annuus*, *Statice* sp. and *Senecio* sp.

Experimental

For each experiment a number of leaves, as nearly uniform in age and appearance as possible, were picked from a single tree. They were placed with their petioles in water and taken to the laboratory as quickly as possible. Unless otherwise stated, fully mature leaves over six months old were used. They were divided into a number of sets, usually 4–6 leaves per set. During cold storage they were kept in open containers. For pre-storage or post-storage at 25° C. they were placed in closed chambers ventilated by an air current and immersed in a constant temperature bath. Leaves were kept in darkness with their petioles in water throughout.

The amount of browning, expressed as a percentage of the total leaf area, was measured by placing the leaves between sheets of glass, illuminating them from below, and tracing their outlines and the configuration of the brown and green areas on sheets of paper. The areas were then calculated by cutting out and weighing the paper.

Development of browning with time

Curves showing the development of browning with time are given in Fig. 1 for loquat leaves picked in 1937 and 1939. The temperature used was 2° C. and the leaves were placed in cold store immediately after picking. Sets were taken out at intervals and post-stored at 25° C. for 24 hr., when the amount of browning was measured. It will be seen that a minimum period at the low temperature is required to induce physiological injury. Leaves exposed to 2° C. for less than this period showed no browning when transferred to 25° C. and kept there for 7 days. The length of the induction period was much shorter in 1937 than in 1939, but the browning when it did develop was more severe in 1939. In general browning only developed after removal from cold store, spreading rapidly for the first 24 hr. and then very slowly. Leaves kept at 2° C. for 20 days already showed a certain amount of browning before removal.

Browning at different temperatures

Sets of loquat leaves from a single picking were placed immediately at −5, 0, 2 and 5° C., and kept at these temperatures for 10 days. They were then transferred to 25° C. and the amount of browning measured after 24 hr. Leaves kept at −5° C.

were killed by the treatment. Browning after exposure to 0, 2 and 5° C. was 57, 42 and 0% respectively.

Effects of pre-storage at high temperatures

A number of experiments were done with leaves of loquat, *Viburnum* and wild fig to determine the effects of high temperature pre-storage on low-temperature browning following exposure to 2° C. Results obtained with loquat leaves in 1937 and 1939 are shown in Fig. 2. In 1937 the leaves were kept at 2° C. for 7 days, in 1939 for 10 days. Pre-storage in both cases was at 25° C. The protective effect of pre-storage was clearly demonstrated.

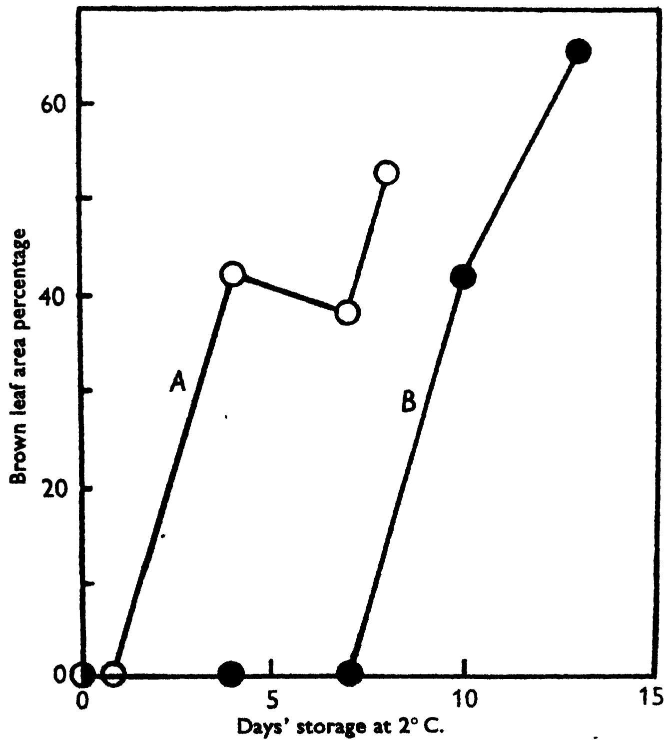

Fig. 1. Progress of browning with time in loquat leaves stored at 2° C. *A*, leaves picked 13 August 1937. *B*, leaves picked 9 June 1939.

A similar result was obtained with leaves of *Viburnum* cold-stored at 2° C. for 30 days (see Fig. 2). In this case laboratory temperature (about 18° C.) was used for pre-storage. An appreciable amount of injury, in the form of darkened areas, became visible during cold storage. Consequently the leaves were not post-stored, the amount of injury being measured immediately after removal from cold store.

Leaves of a wild fig (*Ficus* sp.) picked on 23 June 1939 were stored at 2° C. for 7 days. A control set was kept at laboratory temperature for 7 days and then placed for 7 days at 2° C. Three days after removal from cold store the first set showed

injury equal to 36% of the total area. The control failed to show any injury even when kept for 8 days after removal from store. Seven days' pre-storage at laboratory temperature therefore conferred complete immunity on these leaves.

The relative susceptibility of mature and immature leaves of the loquat was determined in another experiment. Immature leaves developed 91% browning after 7 days at 2° C. followed by 24 hr. at room temperature. Controls kept at room

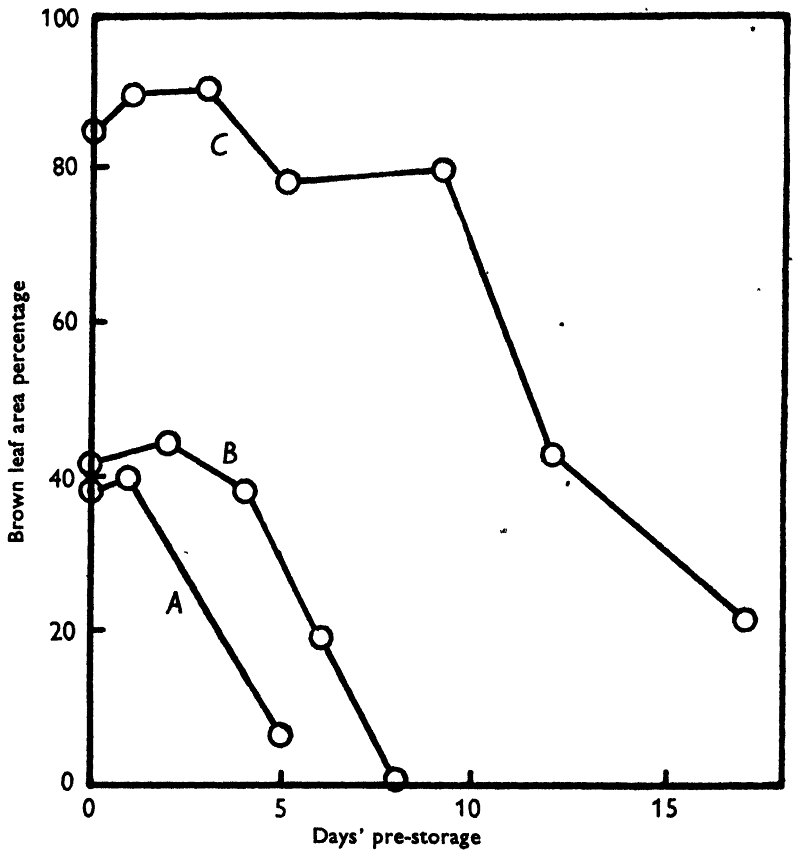

Fig. 2. Effect of pre-storage on amount of browning developed by leaves stored at 2° C. *A*, loquat leaves picked 13 August 1937, kept at 2° C. for 7 days. *B*, loquat leaves picked 9 June 1939, kept at 2° C. for 10 days. *C*, *Viburnum* leaves, kept at 2° C. for 30 days. *A* and *B* pre-stored and post-stored at 25° C. *C* pre-stored at laboratory temperature (about 18° C.).

temperature for 7 days, then placed at 2° C. for 7 days, then transferred back to room temperature for 24 hr., gave no browning. Mature leaves, picked from the same tree at the same time and treated in the same way, gave 20% browning as against less than 1% for the pre-stored controls. It appeared therefore that young leaves were much more susceptible than old leaves to low-temperature injury and responded equally well (or even better) to pre-storage treatment.

Seasonal incidence of susceptibility to low-temperature injury

In the three types of evergreen leaf studied it was found that only during a limited period in the winter were they subject to the form of low-temperature injury described. The actual period of susceptibility varied considerably from year to year. Browning after exposure to 2° C. was not observed during three seasons except in the months June–August. In 1937 it appeared as late as 13 August, but in 1939 it was confined to a period of about 20 days in June.

In Cape Town during the winter atmospheric temperatures are lower (though frosts are practically unknown), sunshine hours less and relative humidities higher than during the summer, and there may be some general relation between these factors and susceptibility to low-temperature injury. But attempts to relate the amount of browning to the meteorological conditions immediately preceding the date of picking were not successful. The phenomena are obviously very complex and numerous "external" and "internal" factors must be involved.

Since immunity is acquired during starvation, it might be suggested that conditions which favour assimilation prior to picking might be an important factor in producing susceptibility. On the other hand, summer conditions almost certainly lead to increased rates of assimilation, and leaves during the summer never showed low temperature physiological breakdown. An investigation of the carbohydrate metabolism of susceptible and non-susceptible leaves might throw some light on the problem, but this has so far not been attempted.

It is interesting that leaves should be more susceptible to low-temperature injury in the winter than in the summer. Meyer (1932) states that "evergreen leaves are not, in general, resistant to cold during the warmer months of the year". But this refers to lethal injury at temperatures below the freezing point of the cell sap. Physiological injury at temperatures sufficiently low, but above the freezing-point, evidently has a totally different seasonal incidence. Kidd & West (1933) have shown in the case of English-grown hot-house tomatoes that "summer-grown fruit tolerates low temperature (5° C.) better than autumn-grown fruit", and Wardlaw (1939) has pointed to the high degree of resistance to cold injury of tomatoes grown under tropical conditions in Trinidad.

Summary

A form of low-temperature browning, or "physiological injury" following exposure to temperatures above the freezing-point of the cell sap, is described for detached leaves of certain evergreen trees.

Leaves picked in the summer are not subject to this form of injury.

Leaves otherwise susceptible may be protected against low-temperature injury by exposing them for a few days to a higher temperature before they are placed in cold store. This resembles the method by which "woolliness" in peaches is controlled in commercial practice.

Attempts to relate susceptibility to low-temperature injury in winter leaves to

atmospheric temperatures, humidities and light intensities at the time of picking were not successful.

The writer held a Carnegie grant during part of the time in which this work was carried out. Thanks are due to the Royal Observatory, Cape Town, and the Government Meteorological Station, Wingfield Aerodrome, for meteorological data supplied.

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ZOOSPORE FORMATION IN A SPECIES OF *STIGEOCLONIUM*

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(With Plate 4)

Introduction

In a collection of plants taken from the dykes near the Thames at Runnymede in October 1937, a few small compact plants were found of an alga at first taken to be a species of *Chaetophora*. After being kept in the laboratory overnight, they were observed to be forming zoospores. It was observed that the filaments producing zoospores were different in appearance from the vegetative filaments, their cells being much longer and larger. Attempts were made to grow the alga in culture, and for this purpose the plants were washed well and introduced into a thin film of agar in a Petri dish and covered with soil solution. For many weeks no growth was observed, and the plants were left for several months, when it was discovered that there were many small plants surrounding the original plants. As soon as these were large enough to handle they were put into fresh agar and have been kept in culture ever since. The following year, more of the original plants were collected from the dykes. Zoospores were obtained from these plants and grown in pure culture so that it was definitely established that the first batch of young plants belonged to the alga originally collected. As the cultured plants became bigger, it became obvious that the alga was a species of *Stigeoclonium*, but so far the exact species has not been identified, due to the fact that the alga shows such varying growth forms.

Method

The alga is cultured in soil solution made up according to the instructions given by F. Mainx (1928). An extract of soil is first made by boiling 1 kg. of soil with 1 kg. of tap water for 2 hr. under a reflux condenser. The apparatus used is made entirely of pyrex glass. The extract is allowed to settle for at least a fortnight before use and is then decanted off and used to make up the following solution: 30 c.c. of soil extract, 200 c.c. of distilled water, 100 c.c. of KNO_3 0·05 % solution. All the water used is distilled in a pyrex glass still. The soil extract can be used for several months if it is sterilized from time to time. When first made it has a *p*H of about 7·5 and tends to become slightly less alkaline with keeping.

Mainx was growing motile organisms like Volvox and he used the soil solution for this purpose. For culturing epiphytic plants, it seemed better to have some solid medium. This was obtained by setting the soil solution with 3 % agar. Plants or pieces of plants are inserted in this agar while it is setting so that they are held

firmly in it. After the agar has set, it is well covered with more of the soil solution. Plants will grow in this medium for several months. The cultures never become contaminated with fungi even though they are exposed to the air. They do, however, collect unicellular algae like Chlorella and some bacteria. Both these can be kept down by pouring off the soil solution and replacing it with fresh once a week.

Zoospores will settle down on the agar and grow into new plants. A culture such as that illustrated in Pl. 4, fig. 1, has been produced in this way. One plant was inoculated into the agar and zoospores allowed to form and germinate. The plants shown are not fully grown. Later they spread sideways and practically cover the agar.

DESCRIPTION OF THE PLANT

The plants collected from the dykes were very small and compact, measuring about $\frac{1}{16}$ in. in breadth. They possessed a very small prostrate system. The cultured plants grow much larger and are not nearly so compact. They develop a much more extensive prostrate system, if the filaments which grow into the agar should be so described. These filaments are thin and elongated and contain only sparse chlorophyll content. They have never been seen to form zoospores. The erect system consists of thicker filaments which are very green and often grow to a considerable length without branching. Most of them end in colourless hairs of from one to several cells. Part of such a hair is seen in Pl. 4, fig. 4. Under certain conditions the cells of the filament may grow out to form branches. The appearance of the alga varies considerably in different cultures.

METHOD OF INDUCING ZOOSPORE FORMATION

As already stated, the material collected from Runnymede formed zoospores in the laboratory before it was used for culture experiments. Material grown in culture does not, in general, form zoospores unless induced to do so. It has so far been found possible to obtain zoospores by a simple procedure. Pieces of old plants are removed from the agar on which they are growing and inoculated into fresh agar so that they are not deeply embedded in it. They are then covered as usual with soil solution. The second morning after inoculation, a large number of the thick erect filaments form zoospores. In the interval one cell division has taken place.

In the summer zoospore formation occurs very early in the morning; during June and July it starts about 6 a.m. If the cultures are placed in the dark room overnight so that they are not exposed to the early morning light, it can be delayed for 2 or 3 hr. and will occur within a few minutes of bringing them into the light any time between 6 and 9 a.m. On the other hand, if the cultures are left longer in the dark, zoospore formation occurs while they are still in the dark, and generally between 10 and 11 a.m. In this case many of the zoospores do not escape from the filaments: instead they germinate *in situ*.

Zoospore formation

During the 24 hr. prior to zoospore formation, most of the cells of the erect filaments undergo a cell division which results in the formation of a transverse cell wall. This wall remains thin in those filaments which are about to form zoospores and does not constrict the filaments as much as the former cross walls have done. Thus in filaments which are about to form zoospores, the cells usually appear to be arranged in distinct pairs. Each pair has a fairly thick wall on either side which is narrower than the maximum width of the filament, while the individual members of the pair are separated by a wall which is only slightly narrower and which is very thin. At this stage the filaments have a very green and turgid appearance. Part of such a filament is seen in Pl. 4, fig. 3. In Pl. 4, fig. 4 another similar filament is seen with part of its hair attached; the transverse division has not occurred in the cells of the hair. Occasionally, before zoospore formation, further cell divisions occur in a longitudinal plane, resulting in filaments which are more than one cell thick. When this occurs, the new cell walls which are formed are even thinner than the previous transverse wall and are difficult to demonstrate until a later stage. Pl. 4, fig. 5 shows the appearance of such a filament after zoospore formation has begun.

The first sign that zoospore formation and discharge is imminent is a gradual elongation and coiling of the filaments. This takes place in jerky movements and suggests that some portion of the cell wall is breaking unevenly, though so far no break has been observed with certainty. Pl. 4, fig. 2 gives a general view of a portion of a whole plant which was forming zoospores. On the left can be seen some of the elongated and twisted filaments; on the right-hand side are a number of filaments which are branched and not forming zoospores. Pl. 4, fig. 5 shows a portion of a filament which is just beginning to elongate and twist. Simultaneously with the elongation of the filaments, and presumably because of it, the protoplast begins to round itself off and withdraw from the cell wall. The filaments therefore contain a number of rounded protoplasts equal in number to the number of cells. Pl. 4, figs. 5 and 6 show the protoplasts just beginning this rounding off process. Fig. 5, it will be remembered, shows the longitudinal as well as the transverse division which may occur in some of the cells and it is due to the extra divisions which have occurred in some of the cells that this filament shows such a lumpy appearance.

The cells now enlarge with great rapidity. This involves an increase both in length and in width. The increase in width occurs in the newly formed transverse walls as well as in the body of the cell. The older cross walls, on the other hand, stretch only slightly; no doubt because of their greater thickness. Each pair of cells becomes somewhat barrel shaped in consequence, while the stretching cell wall that separates the two protoplasts becomes so thin that it is difficult to see it. A slight constriction in the middle of the barrel shows its position. Pl. 4, figs. 7, 8 and 9 show successive stages in the enlargement of the cells, and the rounding off of the protoplasts. Fig. 7 shows an early stage in this rapid enlargement. The irregular

shape of the cells in the middle of the filament is very characteristic of this stage and is due to the fact that the walls do not stretch evenly all over their surface, but begin to bulge in patches in the cells. For example, in the pair of cells marked *x*, the upper cell is bulging on the right-hand side while the lower cell is bulging on the opposite side. The parts of the wall which have not yet stretched show much more clearly than the other parts since the stretching causes the walls to become much thinner. At this stage the protoplasts have not completely rounded off. Events now take place so rapidly that it is impossible to get a photograph of a later stage while the material is still living. Pl. 4, fig. 8, is a micro-photograph of a filament which has been fixed and stained. It shows a stage which follows within a few seconds that shown in fig. 7. The protoplasts are now completely rounded off, the cell walls have stretched further and the pairs of cells are now becoming barrel shaped. Other parts of the walls have now stretched so that at this stage the cells have a more regular appearance. As already explained, the cross-walls other than those formed in the last division do not stretch nearly as much as other parts of the wall. It is these unexpanded cross walls which constrict each pair of cells into a barrel shape. This figure shows these walls clearly. It is not so easy to see the walls which cross the middle of each barrel. Already thinner than the other walls to begin with, they have stretched till they are hardly discernible. Their position can generally be seen by the slight constriction in the middle of each of the pairs of protoplasts. The fixing and mounting has caused a shrinkage in the filament. Pl. 4, fig. 9 shows a filament at a slightly later stage which was photographed after it had been subjected to osmic acid fumes for 10 sec. This stops the process and allows a stage to be photographed without altering in any way the appearance of the filament. The walls have stretched further and, except for the alternate cross walls, are now so fine that they are hardly to be seen. The rounded off protoplasts now only occupy quite a small part of the space within the wall: they are still in pairs, with the unstretched transverse walls showing clearly between one pair and the next. In Pl. 4, fig. 2, long pieces of filament can be seen at *b* in this stage. In the same figure at *a* a piece of filament can be seen before the final stretching of the cell occurs.

Frequently all the cells of a filament, except the hairs, behave in the same way; occasionally a small piece of filament is found in which only a few odd cells are stretching in the manner described. Pl. 4, fig. 10 illustrates this and demonstrates very conclusively the difference in the size of the cell before and after stretching. One cell at the upper end and another cell at the lower end, this latter one just out of focus, have expanded while the cells in between have not altered. These cells were photographed mounted in 0·1 % methylene blue after treatment with osmic acid fumes for 10 sec.

Motility occurs during the final expansion of the cell. The zoospore, formed from the rounded off protoplast, has four flagella. These can be seen in filaments which are mounted in dilute methylene blue. The zoospore begins to move inside the cell, at first by occasional jerking movements, and later more violently when it swims round and round inside the cell. While doing this it frequently hits the cell

wall. Watching the process, one has the impression that this bombardment causes the last stage of the expansion of the cell. It certainly does not initiate it, since the stretching always begins before the protoplast becomes motile. The movements of the zoospores frequently result in the breaking down of the thin cross wall, so that two zoospores may be swimming inside a common boundary. The movements continue until the cell walls give way and the zoospore escapes. The time taken for this to happen varies, and there may be an interval of as much as a minute between the escape of the first and the last zoospore from a filament. In general the process from the beginning of motility to the release of the zoospore takes about 20 sec. The cell walls are so thin by the end of this process that it is difficult to see what finally happens, but their appearance suggests that they are stretched by the bombardment of the zoospore until they finally give way. After all the zoospores have escaped, there is nothing to be seen of the filament except the basal cells still attached to the prostrate system, a few free-floating cells from the apex which include the hair, and a few of the thicker transverse walls scattered round. In some cases, therefore, the original plant is so reduced that there is nothing left but the prostrate system, and within a few minutes the erect system has changed from a mass of inert threads to hundreds of zoospores swimming inside continually enlarging cell walls. In other cases, where zoospore formation has not been so universal, the erect part of the plant has a truncated appearance.

The zoospores are slightly ovoid in shape when they emerge. They swim away rapidly and finally settle down on the agar and germinate very quickly. A number of zoospores have been watched under the microscope during the process and their period of motility has varied between 2 and 3 min. Pl. 4, fig. 11 shows a number of germinating zoospores which are 48 hr. old.

It frequently happens that certain filaments, and occasionally whole plants, do not form zoospores at all after the treatment which is so generally successful. Instead the usual cell division occurs and each new cell proceeds to grow out to form a branch. This is illustrated in Pl. 4, fig. 12. In fact all cases of marked branching have occurred in this way and such plants have been completely altered in appearance. In general this has happened when young plants have been used.

Although the process of zoospore formation here described has been watched in detail only in material grown in culture, where many hundreds of filaments have been observed forming zoospores, it is now obvious that the process which occurred in the original plants after their collection was a similar one. Only the end of the process was seen but it was clearly observed that those filaments which contained the motile zoospores were much longer and larger celled than the other filaments.

Conclusion

There are very few detailed accounts of zoospore formation in green algae and certainly none for *Stigeoclonium*. It seemed worth while therefore to record these observations on zoospore formation and discharge in a species in which the zoospores escape by the rapid stretching of the cell wall and its final disappearance; a method which as far as I know has never been described before.

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EXPLANATION OF PLATE 4

Fig. 1. Photograph of a culture of the alga ($\times \frac{2}{3}$).

Fig. 2. Microphotograph, taken under a Zeiss objective A in combination with a Zeiss ocular $\times$ 10, of a portion of a plant in the process of zoospore formation. The plant was subjected to osmic acid fumes for 10 sec. before the photograph was taken.

Figs. 3–12 are all microphotographs taken under a Zeiss objective D in combination with a Zeiss ocular $\times$ 10.

Fig. 3. Microphotograph of a living filament showing the cell division prior to zoospore formation.

Fig. 4. Microphotograph of a living filament, mounted in 0·1 % methylene blue showing the cell division prior to zoospore formation.

Figs. 5–10 show stages in zoospore formation.

Figs. 5 and 6 are microphotographs of living cells.

Figs. 7 and 9 were photographed after the filament had been subjected to osmic acid fumes for 10 sec.

Fig. 8 was photographed after the filament had been fixed in osmic acid fumes, stained in bismark brown and mounted in glycerine.

Fig. 10 was photographed after the filament had been subjected to osmic acid fumes for 10 sec. and mounted in 0·1 % methylene blue.

Fig. 11. Microphotograph of living germlings 48 hr. old.

Fig. 12. Microphotograph of living filaments showing the branching of all the cells.

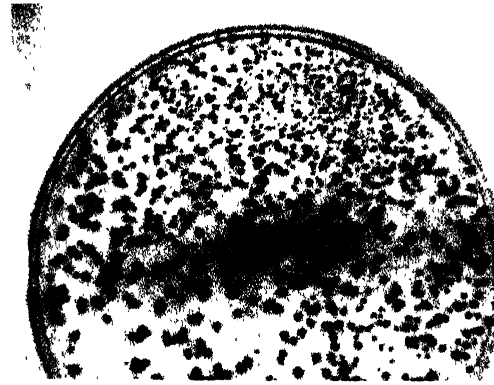

Fig. 1

Fig. 2

Fig. 3

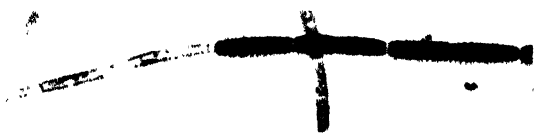

Fig. 4

Fig. 5

Fig. 6

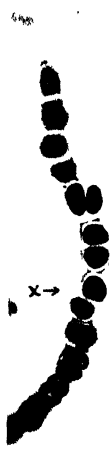

Fig. 7

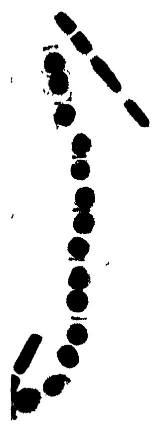

Fig. 8

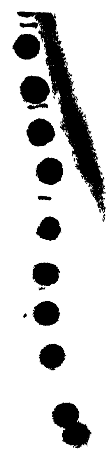

Fig. 9

Fig. 10

Fig. 11

Fig. 12

MADGE—ZOOSPORE FORMATION IN A SPECIES OF *STIGEOCLONIUM*

STUDIES IN THE FUCALES OF NEW ZEALAND

II. OBSERVATIONS ON THE FEMALE FROND OF *CARPOPHYLLUM FLEXUOSUM* (ESP.) GREV. = *CARPOPHYLLUM PHYLLANTHUS* (TURN.) HOOK. & HARV.[1]

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(With 4 figures in the text)

CONTENTS

I. INTRODUCTION

THE members of the Fucoid family Sargassaceae are as characteristic of southern and warmer shores as the Fucaceae are of the colder parts of the northern hemisphere. Largely owing to their inaccessibility the Sargassaceae have been studied comparatively little except for the purpose of morphological and anatomical descriptions and brief accounts in collectors' catalogues such as those of Esper (1802), Turner (1819), Greville (1830), Laing (1899) and Setchell (1925, 1936). Recently a considerable amount of work has appeared on the eastern species (Tahara, Okabe, Inoh). The present opportunity of contributing observations on localized southern species should produce results which, following the classical work of Farmer & Williams and of Nienburg and accompanying that of the Japanese school, may have a wide bearing on general Fucalean and Phaeophycean problems.

The first paper in this series (Delf, 1939*a*) dealt in a general way with several species of the genus *Carpophyllum* Greville, and in particular with the female fronds of *C. Maschalocarpum* (Turn.) Grev. and *C. elongatum* A. & E. S. Gepp. The source of the present species, *C. flexuosum* (Esp.) Grev. and the fixatives employed were the same as for these. Besides the preserved material I have had access to herbarium material direct from New Zealand, as well as in the Natural History Museum, South Kensington (facilitated by the kindness of Mr G. Tandy), and in the Botanical Institute, Berlin (through the kindness of Prof. L. Diels and Prof. O. C. Schmidt).

The species is generally known as *C. Phyllanthus* (Turn.) Hook. et Harv., but

[1] Work approved for the degree of M.Sc. in the University of London.

according to the *International Rules of Botanical Nomenclature* (Briquet, 1935), Art. 60 (1), this name is invalid, Esper's epithet "*flexuosus*" (1802) having priority over Turner's "*Phyllanthus*" (1819). Greville (1830) included the species in his new genus *Carpophyllum* as *C. flexuosum* (Esper) Greville which is the correct name (see Dawson, 1940).

Geographical distribution

Carpophyllum is a characteristically southern genus, and although Okamura (1932) has recorded *C. flexuosum* on the Pacific coast of America, it is practically confined to New Zealand, especially the north and east coasts and the neighbouring islands, as recorded by Laing (1926), who wrote that it is "by no means so common as *Carpophyllum Maschalocarpum* but is generally to be found in deep tidal pools anywhere along the coast" (1899). It thus shares the permanently submerged habit with several other members of Sargassaceae which prefer warmer waters, e.g. *Sargassum*, *Cystophyllum* and *Bifurcaria*, and with *Marginariella*, a New Zealand member of the family Fucaceae.

Treatment

The material has been examined by clearing, swelling, dissecting and by cutting hand and microtome sections. The difficulty of handling material made brittle by long preservation was overcome by using Madge's method (1936) of transferring it from 70 % alcohol to molten 3 % agar solution. The agar sets quickly to a transparent jelly in which objects can be examined and embedded in wax.

Several recommended stains have been tried because of the recognized difficulty of staining seaweeds. The most successful combinations were gentian violet fixed in iodine and counterstained in erythrosin or light green, and Heidenhain's haematoxylin counterstained with light green or orange G. For the staining of young walls in sporelings and at apices Delafield's haematoxylin counterstained with safranin is very satisfactory.

II. MORPHOLOGY OF THE FROND

(i) *General*

In the most recent classification of the family Sargassaceae (Schmidt, 1938) the genera *Carpophyllum* and *Sargassum* are placed in the subdivision Sargassoideae, where the receptacles are specialized, axillary parts of the frond. This material of *Carpophyllum flexuosum* answers to the commonly accepted diagnosis formulated by De-Toni (1895).[1] In addition it supports the systematic value placed by Schmidt (1938) on certain other characteristics, namely, (1) conspicuous tongue cells during development of the conceptacles (as observed by Nienburg (1912), (2) "granular nuclei" (as observed by Le Touzé (1912) and Roy (1938)); and (3) non-mucilaginous, thin-walled vegetative tissue (Le Touzé, 1912). This confirmation is interesting, as the inclusion of further diagnostic features was based on observations of only a limited number of Sargassaceous species.

[1] Fronds differentiated, the vegetative parts forming leafy appendages and vesicles, the receptacles distinct. Three-sided apical cell. Oogonia produce one "oosphere" each.

(ii) *Specific morphology*

Oltmanns (1889) and Gruber (1896) described *C. flexuosum* in detail under the name *C. Phyllanthus*. The vegetative frond is large and luxuriant, reaching a length

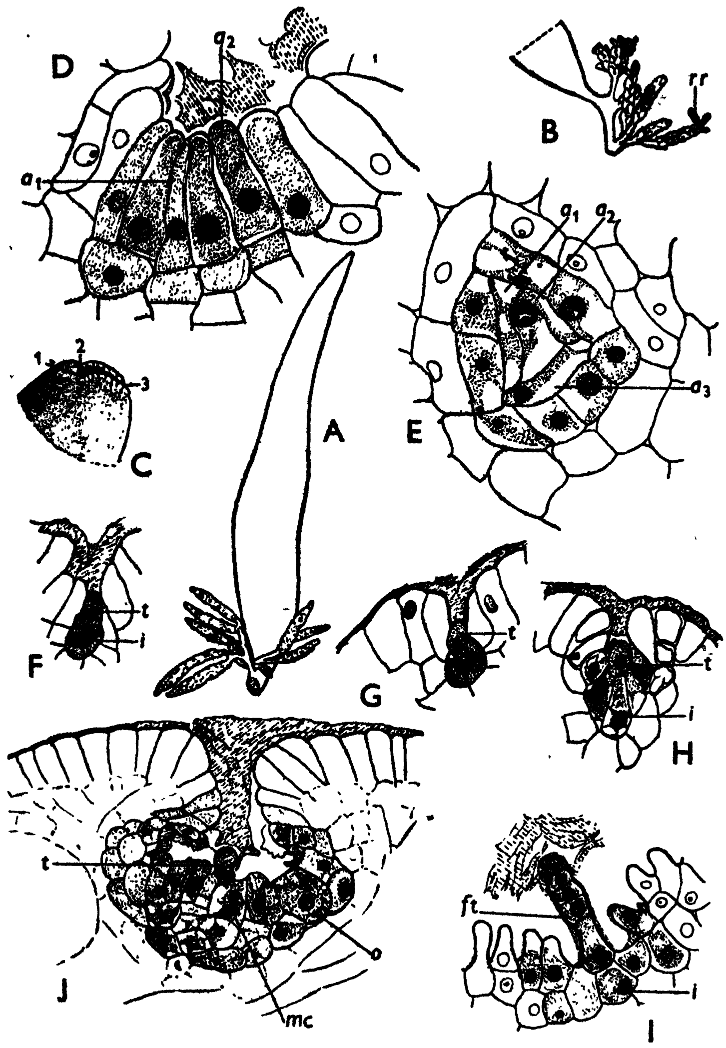

Fig. 1. *Carpophyllum flexuosum*. A, leafy appendage with axillary cluster of receptacles (× $2\frac{1}{2}$). B, receptacles with regenerating tips (*rr*) (× $2\frac{1}{2}$). C, whole apex of receptacle showing angles (1, 2, 3) of apical pit (× 100). D, longitudinal section and E, transverse section of apex, showing the apical cell and its most recent products (a_1, a_2, a_3) (× 650). F, G, H, J, development of the conceptacle *t*=tongue cell; *i*=conceptacle initial; *mc*=oogonial mother cell; *o*=oogonium (× 380). I, filament from the tongue cell (*ft*) (× 650).

of about a yard. The fertile fronds, particularly the male, are more slender and delicate, though more profusely branched. The lateral branches arise alternately from the main axis, forming a monopodial system in one plane (Fig. 4). Each is narrowed at the base and, shortly above this, bears a swollen air vesicle, usually with

a flattened leafy tip which may itself fork. The flattened assimilatory branches on both the fertile and sterile fronds vary in size, unlike those of *C. Maschalocarpum* (Turn.) Grev., which are more uniform.

On those long-ultimate branches which are fertile, the bunches of receptacles are apparently axillary to leafy appendages (Fig. 1 A), which closer inspection shows to be in fact the basal members of condensed branch systems. The inner, younger members of each system are all receptacles, and towards the apex of the frond even the outermost member becomes fertile, by a gradual transition which results finally in the reduction of the fertile branch system to a cluster of two to twelve receptacles unseparated by flattened, sterile tissue.

Although at some seasons leafy appendages are abundant on the fronds of both sexes, they are usually rarer on the male, which is a more spare plant, with the receptacles oftener in pairs than in large clusters. The preserved and herbarium specimens which I have seen strongly suggest that *C. flexuosum* is completely dioecious, although no morphological distinction between the sexes is possible except by careful scrutiny of the surfaces of the receptacles. The male receptacles are translucent even when gorged with sexual cells, and the blistered surface produced by the domed roofs of the receptacles contrasts with the smoother surface of the female.

The Apex. The young vegetative apex consists of a sunken apical cell in a funnel-shaped pit which, when branching takes place, becomes stretched into a slit-like groove towards the new apical cell. This corresponds with the tips of the bilaterally constructed parts in other Fucoids, e.g. the vegetative branches of *Cystoseira abrotanifolia* (Valiante, 1883), and *Sargassum linifolium* (Oltmanns, 1889), and *Pelvetia canaliculata*. The apical pit of the receptacle remains funnel-shaped (Fig. 1 C) and contains in its base an apical cell which is triangular in every transverse section (Fig. 1 E), like that described as long ago as 1876 by Reinke in *Halerica* (= *Cystoseira*) and since then in so many other species of *Cystoseira*, *Sargassum*, *Halidrys*, *Pycnophycus*, *Platylobium* (by Valiante, 1883; Oltmanns, 1889; Hansteen, 1892) that it is safely accepted as a diagnostic feature of the Sargassaceae. In *Carpophyllum* it was first described for *C. Maschalocarpum* (Delf, 1939*a*), and my material of *C. flexuosum* confirms these observations.

Many observers have described the Sargassaceous apical cell as lens-shaped in all longitudinal sections, e.g. Valiante for *Cystoseira abrotanifolia*, Oltmanns for *Halidrys siliquosa*, and Rees (1933) for *Bifurcaria tuberculata*, in which a Brazil nut was suggested as the best analogy for the shape of the cell. In *Carpophyllum flexuosum* vertical sections of the apical cell are far more commonly flattened at both ends (Fig. 1 D) than lens-shaped, and it is possible that the occasional lens-shaped sections are tangential to one of the three angles, and that this cell has the shape of a three-sided truncated pyramid rather than a spindle.[1]

The receptacles. Judging from the condition of material collected at various

[1] Such an interpretation would bring the apical cell nearer to the Fucaceous type which is a four-sided truncated pyramid. Nienburg (1927) has shown that in traumatic proliferations in *Fucus vesiculosus* this is formed secondarily from the three-sided basal cell of a trichothallic hair. Thus the apical cell of *Carpophyllum flexuosum* may be similar to a cell from which the three-sided, Brazil nut-shaped Sargassaceous cell and the four-sided truncated Fucaceous cell could be derived.

seasons, the fertility is seasonal and is at its maximum in the New Zealand summer and autumn, from the end of January until about March.

The receptacles are finger-shaped and rarely more than 5–6 mm. long and 0·5 mm. in diameter. They are usually single, but occasionally forked near the proximal end. Sometimes the tips of old receptacles of both sexes regenerate, growing out to form one or two, or sometimes several, tiny papillae not more than 2 mm. long and 0·25 mm. in diameter, each of which is itself a receptacle (Fig. 1 B) and becomes very densely packed with reproductive cells. The conceptacles of one receptacle are of various ages (see Fig. 2 H). The tip often contains unripe maturing oogonia; the middle region may be clothed with recently shed oogonial contents, and the lower part is spent and translucent. This corresponds with Tahara's observations (1913) on *Cystophyllum sisymbrioides* and two species of *Sargassum*, and Delf's on *Bifurcaria Brassicaeformis* (1935), *Marginariella Urvilleana* (1937) and *Carpophyllum Maschalocarpum* (1939*a*).

Development of the receptacles. The receptacle grows by the activity of a sunken, three-sided apical cell (see p. 286). Its recent products stand out when stained because of their dense cytoplasm and dark nuclei, but when its activity has ceased the pit becomes obliterated so that the tip of the receptacle is quite convex and the cells stain uniformly.

Near the apex one transverse section often includes three evenly spaced conceptacles, so nearly on a level that it looks as if they came originally from three successive products of the apical cell. Usually the receptacle is composed of not more than ten tiers, each of three conceptacles, surrounded and separated by zones of sterile ground tissue. The conceptacles open in the sides of the receptacle by ostioles which, corresponding with the three members of each tier, form three vertical rows (Fig. 2 A, G). The rows continue regularly until the displacement of the conceptacles by mutual pressure during enlargement obliterates the original arrangement.

Judging from the positions of the ostioles on large receptacles cut in serial sections the clockwise spiral initiated by the activity of the apical cell is maintained by the conceptacles (Fig. 2 G). The members of each tier of three are part of this close spiral, so that their ostioles are not usually exactly on a level.

Occasionally, at the extreme base of a receptacle an old cryptostoma is found. It is generally blocked up by a tissue-like tuft of swollen hairs, and strongly resembles an old, spent conceptacle. This observation supports the familiar view that conceptacles and cryptostomata are homologous structures (Bower, 1880; Nordhausen, 1910; Oltmanns, 1922). It was at the bases of receptacles that Sauvageau (1911) found transitions between the two in several species of *Cystoseira*. In *Carpophyllum flexuosum* the conceptacles have another feature common to typical cryptostomata in their forked, slightly pigmented sterile hairs (Nordhausen, 1910 and see p. 290). The cryptostomata seem to arise very early in ontogeny, so that they are found at the bases of receptacles and at the tips of sporelings.

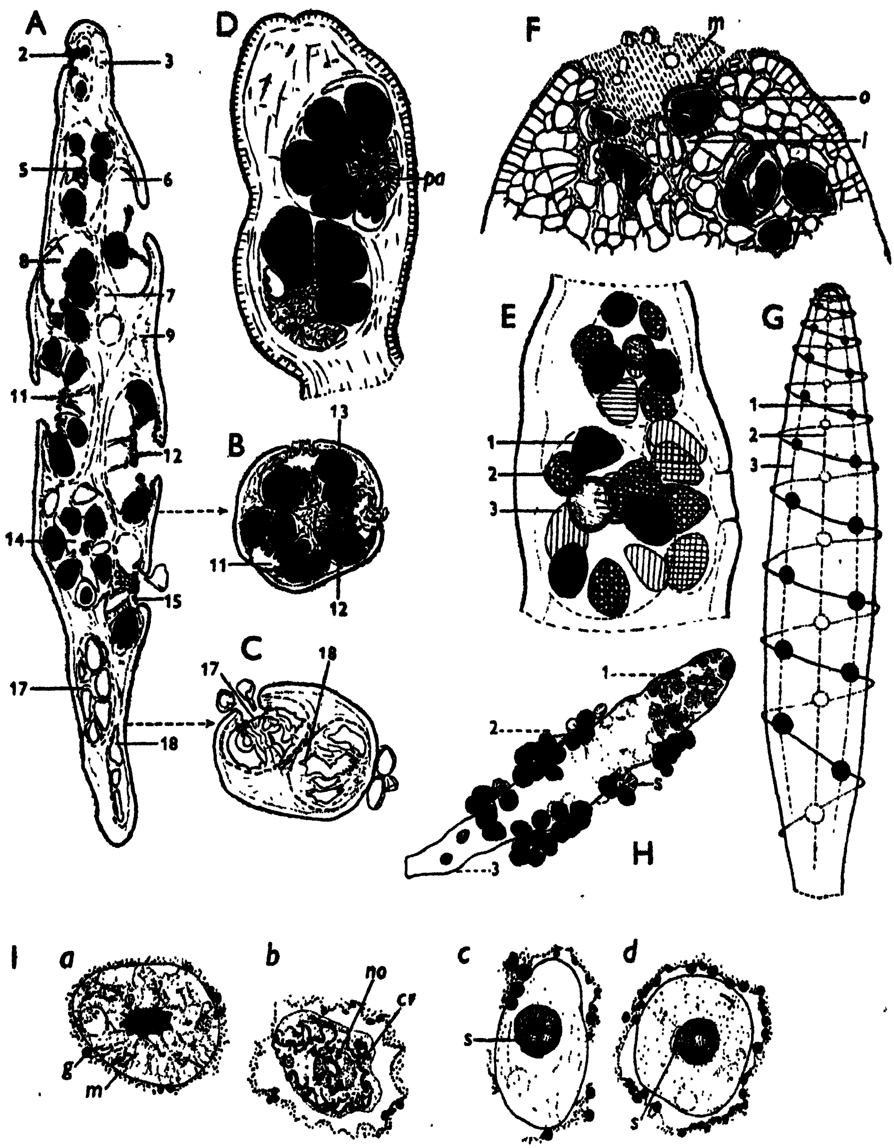

Fig. 2. *Carpophyllum flexuosum*. A, longitudinal section of receptacle cutting two rows of conceptacles. B, C, corresponding transverse sections numbered as part of a spiral (× 50). D, tangential section of receptacle showing closely packed oogonia, and paraphyses (*pa*) at the ostiole (× 70). E, part of cleared receptacle to show arrangement of the oogonia in threes in the conceptacles; 1, 2, 3 = three levels of oogonia (× 70). F, transverse section of injured receptacle with abnormal oogonia (*o*) (× 150); *i* = injured cell; *m* = mucilage. G, diagram of spiral of conceptacles in the receptacle; 1, 2, 3 = three vertical rows (× 30). H, mature receptacle with attached oogonia; 1, 2, 3 = three zones of oogonia; *s* = empty mesochitonous sheath (× 15). I, maturing oogonial nuclei (all × 850); *a*, "resting nucleus"; *b*, nucleus in early prophase; *c*, nucleus with pale nucleoplasm and dark nucleolus; *d*, nucleus with conspicuous "chromophilous spherule"; *cr* = chromatic material; *g* = cytoplasmic granules; *m* = nuclear membrane; *no* = nucleolus; *s* = chromophilous spherule.

III. Development of the conceptacle

(i) *The whole conceptacle*

Origin. After years of discussion and investigation, Nienburg's work on the development of the Fucoid conceptacle (1912), following some preliminary observations by Simons (1906), has superseded Bower's theory; and it is now accepted that a basal cell (conceptacle initial) cut off from a sunken young epidermal cell, is the progenitor of the lining of the conceptacle. The early development in *Carpophyllum flexuosum* corresponds closely with Nienburg's description for members of Sargassaceae, as well as with what Delf has described for the female conceptacle of *C. Maschalocarpum* (1939*a*). Three successive products of the apical cell become separated by two or three more series of cells, and then almost simultaneously sink below the surface, marking the angles of the apical pit. Each sunken cell is the progenitor of a conceptacle. An occasional irregularity is the formation of a "double conceptacle" with a very extensive ostiole and twice as many oogonia as usual; as if two adjacent cells had become conceptacle initials and had then joined forces (as described also by Doubt (1928) for *Halidrys dioica*). The products of the initial are throughout much more densely staining and meristematic in appearance than the adjacent thallus cells. This distinction is useful in showing how the proportions of the ostiole derived respectively from the two sets of cells vary in different species. Bower (1880) used the same criterion, describing the "thin-walled, closely compressed protoplasmic cells, which completely line the cavity. By these peculiarities this tissue is pretty sharply marked off from the adjacent cortical tissue."

A concave wall divides the sunken epidermal cell into a small upper "tongue-cell" and a larger, cup-shaped basal cell, the true "conceptacle initial" (Fig. 1 F).

The products of the tongue cell. As in other members of the Sargassaceae the tongue-cell goes on dividing, but it only exceptionally (Fig. 1 I) produces more than a short, evanescent, mucilaginous filament, which is soon masked by cells growing from the floor of the conceptacle. Hence this relic of trichothallic growth (Nienburg, 1912) is even more vestigial than in most of the Sargassaceae, where a longer filament is common (as noted by Schmidt (1938) and described for *Halidrys* by Nienburg (1912), Doubt (1928) and *Bifurcaria* by Rees (1933)). In contrast, *Carpophyllum Maschalocarpum* (Delf, 1939*a*) and members of Fucaceae (according to Schmidt, 1938) characteristically possess an undivided tongue cell.

The products of the true conceptacle initial. Development is essentially like that described for *Cystoseira barbata* (Nienburg) (Fig. 1 G, H). The cell immediately below the hair may thus, perhaps, be regarded as a "special case" of an apical cell. Its products encroach on the ground tissue, forming the lining of a conical cavity (Fig. 1 J). This lining extends half way up the ostiolar canal, which is about as broad as it is long and is flanked towards the exterior by two or three series of epidermal cells. The participation of these cells in the ostiole is a characteristic which *C. flexuosum* has in common with *Sargassum* (Simons, 1906), *Halidrys siliquosa* (Nienburg, 1912), and *Carpophyllum Maschalocarpum* (Delf, 1939*a*), contrasting

with the species where the lips are formed from epidermal cells only, namely, *Halidrys dioica*, *Cystoseira*, *Pelvetia*, *Ascophyllum*, *Bifurcaria*, *Fucus*.

From the inner rim of the ostiole short papillae grow out to produce unbranched hairs (Fig. 3 A) which, by analogy with the similarly formed but longer hairs in other Fucoids, are called periphyses.

The central column and paraphyses. Simultaneously the floor cells, continuing division in the transverse and vertical planes, form a central column about seven cells in diameter (Fig. 1 J). The ultimate cell in each series forms a papilla which usually divides vertically so that the central column terminates in a wide brush of slightly pigmented forked hairs, surrounding the initial filament and called by analogy with other Fucoids the paraphyses (Fig. 3 A). A few similar series of cells separate the oogonia (Fig. 2 B–D).

Branching hairs are very rare in female conceptacles. In the form of antheridial hairs they occur in monoecious species such as *Sargassum Filipendula* and *Bifurcaria tuberculata*, but Bower (1880) already noted that "no branching occurs in the neutral hairs of the female conceptacle". Their appearance in *Carpophyllum flexuosum* as well as in a few other dioecious Fucoids such as *Himanthalia lorea* (Wille, 1910) and *Fucus serratus* (Nordhausen, 1910) may possibly be explained by regarding them as homologous with the antheridial hairs. When retained after separation of the sexes they appear to carry out only their secondary, secretory function (see p. 297).

The sterile centre of the floor is itself noteworthy. It is not mentioned specifically in any descriptions of Sargassaceous conceptacles except that of *Carpophyllum Maschalocarpum* (Delf, 1939*a*), nor is it figured except perhaps in *Sargassum Kjellmanianum* by Inoh (1930). It resembles most closely the proliferations from the floors of the cryptostomata in a salt-marsh form of *Fucus ceranoides* (Skrine *et al.*, 1932). The interpretation which these workers suggest is one to which I had come independently, namely, that the central block may be formed by the "congenital growth of a group of hairs". This further supports the view advanced above that the conceptacle is a special case of an apex, for these workers show how a cryptostoma resembles an apex both in originating from one sunken superficial cell and in forming hairs from the lining cells of the pit. The difference is merely that in an apex the initial cell's activity proceeds unchecked, while in a cryptostoma and in a conceptacle it is only responsible for the lining; and the hairs which grow out from this, normally apart but sometimes concrescent, are the final product.

(ii) *The oogonia*

Development. When the papillate central block has been established, the first oogonia are initiated in the groove between the floor and the sloping wall (Fig. 1 J, *o*). A cell lining the conceptacle swells, becoming the oogonial mother cell, and its nucleus divides (Fig. 1 J, *mc*). Cell division below the inner surface of the conceptacle follows, so that the stalk cell is completely embedded and non-functional. So far as I can at present judge this always takes place, as in *Sargassum* (Kuneida, 1928; Nienburg, 1910), contrasting with *Carpophyllum Maschalocarpum* (Delf,

1939*a*), where the cutting off of a stalk cell is inconstant. The cell is flat and soon becomes indistinguishable.

Examination of whole cleared receptacles and of young transverse sections suggests a spiral arrangement of the oogonia within the conceptacles, the younger ones being at a deeper level, farther from the ostiole (Fig. 2 E). Closer inspection shows that the oogonia are laid down in twos or threes, separated only by thin plates of cells. Two sets seem to be initiated first, sometimes followed later by one or more extra batches of three. Such regularity of the organization within the conceptacle makes it plausible that the "conceptacle initial" behaves as a specialized apical cell until the whole lining has been laid down; like the apical cells of the cryptostomata described by Skrine (cf. p. 290). If this is a three-sided cell then the formation of the oogonia in successive threes is readily intelligible. The average number of oogonia is nine, as counted in a hundred conceptacles taken at random; but there are often only six, for example in the small regenerated receptacles (see p. 287). The conceptacles in the full-size primary receptacles may have as many as sixteen oogonia, but even these are very few compared with the innumerable small ones in the large conceptacles of Northern Fucoids.

The oogonia become ovate through mutual pressure (Fig. 2 D) and their enlargement displaces the mucilaginous cells around them so that the central column becomes indented and stellate in transverse section and the paraphyses which, unlike *C. Maschalocarpum*, are present between the oogonia often appear to grow from the outside of the oogonial wall itself (Fig. 3 A). During all this swelling the cavity naturally does not enlarge proportionately with the rest of the conceptacle, and the diaphragms of ground tissue between the conceptacles become so thin that it is difficult to tell where one conceptacle ends and the next begins (Fig. 2 B).

The cells exposed to the cavity of the conceptacle, and especially the hairs, secrete densely staining stratified mucilage whose function is considered below (p. 297). Its presence is characteristic of Fucoid conceptacles and was connected with the activity of forked hairs by Nordhausen and by Wille (see p. 297).

Cytological maturation of the oogonium. The oogonia almost finish enlarging inside the conceptacles. When nearly ripe the long axis averages 150μ, and the short axis 110μ. By the time nuclear maturation is completed, usually immediately after extrusion, the long axis has increased to 178μ, and the short axis to 137μ, involving an approximate doubling in the volume, which corresponds with what happens in *Fucus* (Strasburger, 1897) between the uninucleate and eight-nucleate stages. The oogonia are about half as large again as those of *Carpophyllum Maschalocarpum*, and larger than those of *Coccophora* and *Turbinaria*, but smaller than the oogonia of several species of *Sargassum* (Inoh, 1930). Inoh attributes significance to the size, correlating it positively with the number of "primary rhizoids" in the embryo, and with the level of advancement reached by the species. I hope to consider this question more fully in a later paper on embryogeny.

In the nearly ripe oogonium a layer of palely staining cytoplasm separates abundant food-reserves and pigment from a large central nucleus with a palely

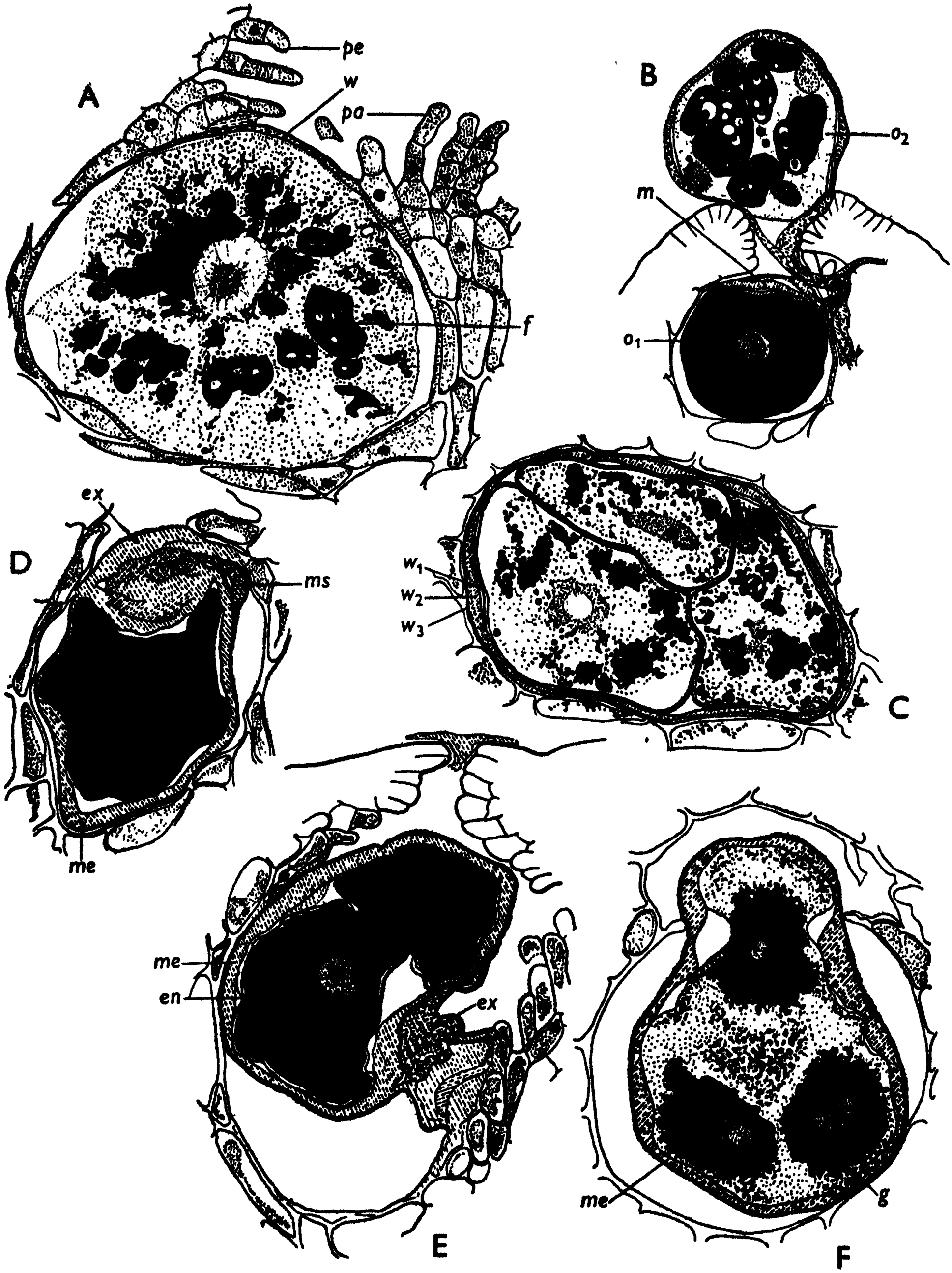

Fig. 3. *Carpophyllum flexuosum.* A, longitudinal section of maturing oogonium (× 330) with undifferentiated wall (w): f = stored food; pa = paraphyses terminating column of concrescent hairs; pe = periphyses. B, longitudinal section of conceptacle with uninucleate oogonium (o_1) inside and stalked eight-nucleate oogonium (o_2) extruded; m = mucilaginous mesochitonous caps (× 180). C, abnormal oogonium with four cells (× 330); w_1 = wall of one cell; w_2, w_3 = layers of oogonial wall. D, stalk formation: ms = mesochitonous tag pressed to side; ex = exochiton; me = mesochiton (× 330). E, beginning of emergence of oogonium; complex stalk not yet lengthened (× 330). F, escaping eight-nucleate oogonium; three nuclei cut in section, each surrounded by dense granules (g) (× 330).

staining nucleolus and finely granular cytoplasm (Figs. 3 A, 2 I, *a*). This type of nucleus, as was mentioned in the diagnosis of the Sargassaceae (p. 284), is regarded as typical of the family. Very shortly before the extrusion of the oogonial contents (see p. 294) cytological maturation begins. The very limited number of stages in maturation and the resemblance between the oogonia in one conceptacle suggest that they are shed periodically, as in *Sargassum* (Tahara, 1909). This is supported by the clear macroscopic delimitation of three zones in the receptacle (see p. 287), one at a time shedding its conceptacular contents. Hence I have been unable, without fresh material, to trace the complete cytological sequence. The stages which I have found are interpreted by the help of comparison with earlier descriptions, especially those of Farmer and Williams and the Japanese workers. Although different fixatives produce certain discrepancies between the two batches of material of *Carpophyllum flexuosum*, some of my figures are so like those of other observers that it seems safe to assume correspondence.

Cytological change begins with the emergence of scattered granules from the nucleoplasm matrix, which previously stained very faintly (Fig. 2 I, *b*). Very little chromatin appears and the most striking structure in the nucleus, through what seems to be a very long period, is the nucleolus. Finally the only conspicuous nuclear object beyond this is a single or double, rod-shaped or spherical body close beside it (Fig. 2 I, *c*, *d*) which takes up chromatin stains strongly. The proximity of the two suggests that the dark granule becomes incorporated in the now heterogeneous nucleolus. The oogonial contents seem commonly to be extruded at this stage. In the absence even of direct evidence of spindle formation, the next stage to be seen is the eight-nucleate result of maturation.

Typically in Fucoids the oosphere nuclei are the products of a division which follows meiosis; for which there are several pieces of indirect evidence in *C. flexuosum*. The series of early changes strongly suggests the protracted progress of meiotic prophase, and walls are not normally formed after the separation of the nuclei (but see below, p. 295). Above all, the darkly staining body near the nucleolus (Fig. 2 I, *c*, *d*) is significant for, in its shape, size, position and staining reactions, it recalls a body first noted by Williams (1904) in the tetrasporangium of *Dictyota* and called by him a "chromophilous spherule", which was peculiar to the reduction division. Georgevitch (1918) and Carter (1927) found a comparable body in *Padina Pavonia*. In the nearer relatives of *Carpophyllum*, Tahara (1929 *b*) described a dumb-bell-shaped spherule in *Coccophora Langsdorfii* and Okabe (1929) in *Sargassum Horneri*. All the chromophilous spherules described agree in being confined to heterotypic prophase. They have been accorded little comment and are chiefly significant at present in indicating meiosis. More detailed cytological investigation of them would probably be amply repaid.

The elusiveness of the post-prophase spindle formation may possibly be accounted for by an unusual kind of nuclear division which has been described by Geitler (1930, 1935) for species of *Spirogyra* and by Svedelius (1937) for *Lomentaria rosea*. Here the chromosomes actually migrate into the nucleolus and divide there, so that nuclear division is accomplished by its constriction instead of by normal

spindle formation. The absence of this kind of division in related species does not rule it out for the meiosis of *Carpophyllum flexuosum*, since Geitler showed that even within the genus *Spirogyra* some species had this and some division of the normal kind.

Time at which oogonial maturation takes place. From the rarity of completely matured oogonia within the conceptacle (cf. p. 295 and Fig. 3 B) it seems likely that maturation begins immediately before the oogonial contents are shed. Thus some partially evacuated conceptacles contain large oogonia with their nuclei in prophase, while other oogonial contents which have recently escaped possess stalks connected to the interior of the same conceptacle, and are already eight-nucleate (see Fig. 3 B).[1]

Fig. 4. *Carpophyllum flexuosum*. Herbarium specimen of female frond, collected August, 1936 (× ⅛).

Similar observations are recorded for *Cystoseira barbata* (Nienburg, 1910); *Cystophyllum sisymbrioides* (Tahara, 1913); *Sargassum Horneri* (Tahara, 1913; Kuneida, 1928; Okabe, 1929); and *Bifurcaria tuberculata* (Rees, 1933). In these species maturation advances considerably before extrusion, so that a well-organized single oosphere escapes. It appears, however, that *Carpophyllum flexuosum* must resemble even more closely a few species where maturation is only actually completed outside the conceptacle, namely, *Sargassum linifolium* (Nienburg, 1910); *Coccophora Langsdorfii* (Tahara, 1929 *b*); and *Marginariella Urvilleana* (Delf, 1937). Delf correlates this late maturation partly with the formation of a single oosphere from an oogonium, contrasted with most of the Fucaceae where eight oospheres are organized much longer before shedding; and shows (1935, 1937) that such maturation is apparently confined to a few deep-water Fucoids whose oogonia become attached by mucilaginous stalks (see p. 296).

[1] The extruded oogonial contents cannot therefore be called either an "attached oogonium" or an "oosphere", but compose a syncytium (see footnote to p. 295).

Abnormal oogonia. Very rarely unshed oogonia are found deeply embedded in otherwise spent conceptacles, and containing eight scattered nuclei (cp. Fig. 3 F). Their position has probably caused their accidental retention after maturity, as in exceptional oogonia of *Sargassum Filipendula* (Simons, 1906). The completion of maturation here shows that the process, though usually accompanying it, is not dependent upon escape from the conceptacle.

Of greater interest is the injured region of one receptacle, where each oogonium contains four uninucleate cells (Fig. 2 F). These are walled and round off from one another (Fig. 3 C). They are paralleled by an abnormality in *Carpophyllum Maschalocarpum* (E. M. Laing, 1939, unpublished), and in *Sargassum Filipendula*, where Simons found "one oocyst...which contained two eggs and two oocysts which contained eight eggs", apparently as the result of wounding. Outside the conceptacle I have found a few comparable attached oogonia where each sheath contains from two to eight uninucleate oospore-like cells instead of a syncytium.

It is tempting to speculate on the significance of these freaks, especially in relation to the theory of traumatic reversion.[1] All the abnormalities combine to show the complete and lasting equivalence of several "oosphere nuclei" which seem able to develop parthenogenetically, or possibly as a result of multiple fertilization. Members of the Sargassaceae capable of behaving in this way cannot be very far removed from the Fucaceae where two, four or eight oospheres develop equally.

IV. Extrusion of the oogonial contents

(i) *Morphological basis*

The young oogonium in all members of the Fucales is a simple structure in which a single wall surrounds a uninucleate protoplast. This is true of *Carpophyllum flexuosum*, where, however, the wall is fused except at its apex with the walls of the surrounding cells which line the conceptacle (see Fig. 3 A). Just after the beginning of cytological maturation the wall assumes a more complex structure. The names used for the layers are those originally applied to *Fucus* by Farmer and Williams (1898). The exochiton is the first to become distinct, and the endochiton is usually not clearly marked until after extrusion. These two layers function essentially as in typical Fucoids. The mesochiton is thicker than either, but histological tests similar to Resühr's (1935) on *Fucus* have failed to reveal its differentiation into the multiplicity of layers which he found enclosing each packet of shed oospheres.

In function the mesochiton is like that of some deep-water species investigated by Delf whose solitary oospheres[2] become attached by gelatinous stalks, e.g. *Sargassum, Cystophyllum, Bifurcaria* (1935 and later) (see p. 296). It becomes

[1] The walled cells within the conceptacle are open to an interpretation other than parthenogenesis, supported by their arrangement like Dictyotalean tetraspores. Wounding may have caused reversion to a less condensed type of life cycle than that of the Fucales. In this case the surviving gametophytic division would be postponed until after emergence of the "spores". The requisite homology of the oogonium with a unilocular sporangium is upheld by the complexity of the sporangial wall in *Asperococcus* (M. Knight, unpublished) which agrees with the early differentiation of the oogonial wall in *Carpophyllum*.

[2] The term "oosphere" is used for the syncytial contents of the oogonium, really equivalent to eight oosphere initials.

thickened apically by the deposition of a few darkly staining but ill-defined mucilaginous layers which form a large hump between the protoplast and the exochiton (Fig. 3 B), recalling Kuneida's figure of the gelatinous cap in *Sargassum Horneri* (1928). A ring-shaped gap often forms between the exochiton and the side of the hump, appearing as an ear-shaped space on either side in longitudinal section. Swelling the walls in 2·5 % potash and then staining in safranin or methylene blue differentiates the mesochiton and endochiton. The former swells and wrinkles, and becomes stained while the latter remains pale and soon disintegrates.

As its volume increases the end of the pad of mucilage slides down between the exochiton and the future oosphere, tending to indent one side of this (Fig. 3 D). The pad, presumably being sticky, adheres to the exochiton at one side, generally the side lying against the central column. Meanwhile its continued swelling bursts the exochitonous cap, making an opening into the cavity of the conceptacle (Fig. 3 E). The doubled loop of the lengthening stalk is probably the first part to emerge into the cavity, so that the oosphere, enclosed in mesochiton, is pulled out backwards. When it is clear of the exochiton the stalk probably unfolds, so that the original apex becomes the base, and the oosphere can now be pushed through the ostiole whilst remaining attached securely to the side of the central column.

A cross section at this stage shows a solid, apparently homogeneous stalk continuous with the thin wall which encloses the oosphere (Fig. 3 B). The oogonium of *Carpophyllum* thus differs from that of a typical Fucaceous species in which the mesochiton enclosing the oospheres is wholly shed into the water in a mass of mucilage. In its loss of this measure of independence and in its high degree of specialization the oogonium of *Carpophyllum flexuosum* falls in line with that of a few members of Sargassaceae in which mesochitonous stalks have been described, notably *Cystophyllum sisymbrioides* (Tahara, 1913); *Sargassum Horneri* (Tahara, 1913; Kuneida, 1928); other species of *Cystophyllum* and *Sargassum* and of *Bifurcaria* (Delf, 1935) and *Carpophyllum Maschalocarpum* (Delf, 1939*a*). From Tahara's account both species of *Carpophyllum* must agree closely with *Sargassum*, where an apical cap of jelly spreads down between the exochiton and the oosphere forming a stalk, and the exochiton then bursts so that the oogonium is swung out, its apex becoming its base. The fuller descriptions given by Delf corroborate this early work but reveal differences in detail between the several genera. The stalk adheres, for example, to the inside of the broken exochitonous cap in *Bifurcaria*, but lower down in the exochiton of *Carpophyllum*.[1]

(ii) *Mechanism of extrusion*

Working from this morphological basis and from comparison with allied algae it is possible to suggest some features of the mechanism of oogonial extrusion. Since the sexual cells appear to escape similarly from the well-known emergent Fucoids

[1] Two species of *Marginariella* (Delf & Hyde, 1936; Delf, 1937) are also found to possess oogonial stalks, but these seem to be formed by definite splitting of the mesochiton into layers. Since *Marginariella* is often classed in the family Fucaceae (Oltmanns, Svedelius (1911) and Schmidt) these two distinct methods of achieving oogonial attachment may indicate parallel evolution in members of Fucaceae and Sargassaceae exposed to similar conditions.

of the north and the permanently submerged, mainly Sargassaceous species of the south, the universal influence of tide variations is likely to play a part. As Delf (1935) suggests: "The periodic changes in depth brought about by ebb and flow must also involve changes in the intensity and nature of the light transmitted, as well as in the hydrostatic pressure of the overlying water." The direct influence of such changes in hydrostatic pressure upon the fronds of seaweeds has actually been demonstrated recently by Damant (1937) working with *Ascophyllum*. A relationship between Sargassaceous extrusion and tidal fluctuations has been shown by Tahara for *Sargassum* (1909, 1913) and *Turbinaria* (1929*a*), in which all the plants in one locality ripen simultaneously. The eggs are usually shed in three batches, at fixed intervals after the highest spring tide. The three ages of conceptacle in a receptacle (Fig. 2 H) and the uniformity of the oogonia in each would thus be consistent with similar periodicity in *Carpophyllum flexuosum*.

The most fertile period of the species is December–January, when low tides in the neighbourhood are generally at night, and high tides in the early morning (Spender, 1932). Abe (1938) and others have found that Sargassaceous sexual cells are often shed at dawn, perhaps because of the altered illumination. If this is true of *C. flexuosum*, then the oospheres must be liberated with the increasing hydrostatic pressure of the rising tide. More light would be likely to accelerate the assimilation of the pigmented cells terminating the central block in the conceptacle, and thus to raise the turgor of these cells. Schreiber (1930) showed for *Fucus* that such turgor changes, due to alterations in the concentration of solutes, helped to force the sexual cells out.

External pressure changes would have their effect on the abundant mucilage secreted mainly by the central block of forked hairs. Such "slime" has been shown already by Nordhausen (1910) and Wille (1910) to help in expulsion in *Fucus* and *Himanthalia*. Thus various factors, internal and external, would combine to raise the pressure in the conceptacle and force the oospheres out via the already slippery ostiole. Once the escape of one or two oogonia has secondarily enlarged the cavity, the central block probably becomes pushed to one side or the other, leaving a clear passage for one oogonium, while blocking the opposite side and preventing congestion in the ostiole. The latter, at first no wider than an oogonium, soon becomes permanently and greatly extended. Inside the conceptacle the remains of exochiton and mesochiton swell up and the ground tissue also becomes loose and gelatinous (Fig. 2 C) so that the blocked cavity comes to resemble an old cryptostoma.

Receptacles at this stage are easily recognized by being clothed with dark bands of ripening oogonia (Fig. 2 H), attached by firm stalks which are composed mainly of mesochiton reinforced by exochiton at the attached end. The mesochiton swells especially at the apex and becomes very thick but translucent. The toughness and firm attachment of the stalks is proved by their survival of the journey from New Zealand, and of years in a herbarium.

V. FORMATION OF THE OOSPORE

After extrusion the endochiton becomes clear and nuclear maturation, passing quickly through the bi- and quadri-nucleate stages, yields an eight-nucleate oogonium. The nuclei remain in pairs at the periphery for some time and then become more evenly distributed, and surrounded by definite aggregations of granules very like those seen in *Cystophyllum*, *Sargassum* and *Turbinaria* (Tahara).

Many of the extruded, attached oogonia contain only a single, large nucleus surrounded by radiating granules, and in this condition possess strongly resistant and darkly staining membranes absent in the eight-nucleate stage. This evidence and the constant co-existence of ripe "male" and "female" fronds indicates that the normal life cycle involves fertilization, and that these attached bodies are oospores.

The high degree of elaboration reached by each of the "oosphere initials" (cf. Fig. 3 F) suggests that their behaviour may resemble that of the oospheres of *Sargassum tortile* (Abe, 1938), *S. Horneri*, *S. enerve* and *Cystophyllum sisymbrioides* (Tahara, 1913; Tahara & Shimotomai, 1926), where one peripheral nucleus is fertilized in the presence of seven other equally vigorous gametes. The potentiality of more than one until the moment of fertilization has been shown by Abe who occasionally saw two fertilized, and by Tahara (1927) who induced artificial parthenogenesis of several sister oospheres in one oogonium of *Sargassum*. My observations on *Carpophyllum flexuosum* agree with those of Delf on *C. Maschalocarpum* in leading to the tentative conclusion that the supernumerary oosphere nuclei do not degenerate before fertilization. In this respect *Carpophyllum*, *Sargassum* and *Cystophyllum* diverge from *Coccophora* (Tahara, 1929*b*), *Cystoseira* (Nienburg, 1910) and *Bifurcaria tuberculata* (Rees, 1933) where one nucleus becomes large and central and the others are extruded peripherally before fertilization.

Finally the seven extra nuclei in *Carpophyllum flexuosum* appear to degenerate simply by being reabsorbed *in situ*, like those of *Sargassum* (Nienburg, Abe). In many attached oospores, from one to seven palely staining traces of chromatic material are scattered between the large central nucleus and the periphery. They are not found outside the cytoplasm, which is the composite product of eight oospheres. This type of oogonium contrasts with that of *Cystoseira* where one oosphere is strongly dominant. As Smith (1938) suggests, both types may be traceable to the less specialized, Fucaceous course of development.

Interpretation of the oogonium

The familiar use of the term "oogonium" does not necessarily imply homology, for the oogonia of other algae are usually direct gametangia on haploid plants. Biologically this organ in the Fucales was recognized already by Strasburger (1906) as formed by convergence of the tetrasporangium and gametangium. The same interpretation is adopted by modern authorities such as Taylor (1922), Oltmanns (1923) and Tilden (1935), admitting that the oogonium is the seat of meiosis, and therefore is a "tetrasporangium". Hence the first four nuclei in it are "megaspore nuclei", only exceptionally separated by walls. As Strasburger emphasized, the

increase in size of the protoplast and the pause before the third division, at least in *Fucus*, point to a difference in homology between this and the preceding divisions. The last yields eight haploid nuclei, so that the anomalous oogonium in the "female" plant of the Fucales may be regarded as a "megasporangium" producing a syncytial gametangium equivalent to four naked, two-celled gametophytes.[1]

The brief identity of a gametophytic phase is supported by the pairs of nuclei which remain near the periphery before separating into "oosphere initials". The completion of maturation outside the conceptacle may be regarded as another slight degree of gametophytic independence (see Delf, 1939*b*).

VI. Embryogeny

The stalked oogonial contents remain attached for a period which probably, from analogy with similar members of the family, lasts for several days. Embryogeny now begins inside the sheath which is reinforced by a thick, deeply staining oospore membrane, resistant to the action of caustic potash. Normally a multicellular sporeling develops, with a tuft of sixteen equal rhizoids derived from a lens-shaped rhizoidal cell, and the dissolution of the apex of the mesochiton releases the embryo from its anchorage. Exceptionally, either through parthenogenesis or through multiple fertilization, two to eight oospores develop simultaneously in one sheath (see p. 295), each usually producing one centrifugal rhizoid.

The embryological details of this species are very interesting and may have some significance when compared with the Japanese work on Sargassaceous sporelings which has appeared in recent years. The morphology and anatomy of the older sporeling too are striking, and on the evidence of recapitulation may throw some light on ancestry. I hope to make the consideration of a few embryological problems in the family the subject of a sequel to this paper.

VII. General considerations

A feature that stands out from this account of *Carpophyllum flexuosum* is the high level of reproductive precision reached by the "female" frond. The conceptacles are not simply sunken cavities but show division of labour, the centre of the floor producing a sterile column of concrescent hairs, while the rim produces a few large oogonia.

The species achieves considerable specialization for cross-fertilization. This is first ensured by the complete dioecism of the fronds, and then provided for by the attachment of the extruded oogonial contents to the conceptacles. Anchorage is

[1] The many layered oogonial wall strongly recalls the sporangial wall of *Asperococcus* (see above, p. 295), which even has a specially thickened apex in the middle layer. This supplies further proof of the homology of the oogonium with a unilocular sporangium. An additional deduction from the complex wall may be tentatively suggested: that only the outer layers are truly sporangial, the much thinner and later developed endochiton being actually the last remains of an independent gametangial wall comparable with what is found in *Laminaria digitata*. Gardner's observations (1910) on Fucoids with less than eight functional sister oospheres show that the nuclei of the suppressed oospheres are extruded between the endochiton and mesochiton in *Hesperophycus* and in *Pelvetia fastigiata*, suggesting a real difference between the endochiton and the outer layers of the wall.

effected by a modification of the middle layer of the oogonial wall, and is accompanied by the reduction of the finally functional oospheres to one, although the others develop equally until the point of fertilization. The evolution of the stalked, solitary "oosphere" in dioecious Fucoids seems to be correlated with their habit of growth in comparatively calm water, where they are never exposed, and where completely freed sexual cells would be fated to sink too soon. Delf (1935) has traced this correlation in a number of species of *Cystophyllum*, *Sargassum* and *Bifurcaria*, and her conclusions are now borne out by *Carpophyllum*. The sporelings also benefit by the stalks and become multicellular before quitting their position of security.

VIII. SUMMARY

1. The female fronds of *Carpophyllum flexuosum* (Esp.) Grev. (better known as *C. Phyllanthus* (Turn.) Hook. & Harv.) have been examined as a contribution to the series of papers on the Fucales of New Zealand.

2. The species is typically Sargassaceous, and the "male" and "female" fronds are practically indistinguishable.

3. (*a*) Examination of the three-sided apical cell in vegetative and reproductive tissues suggests that it is a truncated pyramid rather than a spindle.

(*b*) The activity of the apical cell results in trimery in the receptacles, the conceptacles being in a spiral disposed in three vertical rows.

4. The conceptacles develop by the activity of a sunken epidermal cell after whose first division the basal "initial cell" acts as an apical cell, while the upper "tongue-cell" forms a short filament.

5. The centre of the floor of the conceptacle is occupied by a sterile column produced by the concrescent growth of a group of hairs, whose ends are branched and may therefore represent the antheridia of a monoecious ancestor.

6. (*a*) The oogonia appear to be formed in the conceptacle in threes, in a spiral. The sunken initial cuts off a stalk cell and then swells but remains uninucleate until extrusion.

(*b*) Maturation of the oogonium begins before extrusion, and meiotic prophase is indicated by the appearance of a peculiarly darkly staining spherule in connexion with the nucleolus.

7. Exceptionally, four cells are formed within the unshed oogonium in injured conceptacles. Comparable abnormalities occur outside, when two to eight oospores develop instead of a solitary one. These abnormalities are taken as indicating reversion to an ancestral, Fucaceous type of oogonial contents.

8. The oogonial wall becomes differentiated into three layers. The mesochiton forms a stalk by whose unfolding the oogonial contents are extruded and anchored. Some of the factors controlling this extrusion are considered.

9. After extrusion, cytological maturation seems to be completed quickly, and the seven supernumerary nuclei only degenerate after fertilization, by disappearing in the cytoplasm.

10. The "oogonium" is interpreted as a megasporangium producing a syncytial gametangium equivalent to four two-celled gametophytes.

11. A multicellular sporeling of the *Sargassum* type develops within the mesochitonous sheath.

12. The permanently submerged habit in calm water is accompanied by well-developed reproductive economy, which includes adaptation for cross-fertilization, and the formation of solitary, attached oospores, which begin their germination in close relation to the parent plant.

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SUBMERGED PEAT AT SOUTHAMPTON

DATA FOR THE STUDY OF POST-GLACIAL HISTORY. V

By H. and M. E. GODWIN
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(With 3 figures in the text)

It has been known for a long time that fresh-water deposits are submerged in the estuary at Southampton (Fig. 1), and extensive peat beds have been described during excavations of the numerous great docks on this Harbour. In March 1932, through the kind agency of Mr O. G. S. Crawford and Mr H. G. McHaffie, we were allowed to examine the last remaining section of the peat beds cut through in digging out the new (George V) Graving Dock of the Southern Railway. Our field notes gave the profile shown in Fig. 2. Similar deposits had been already described

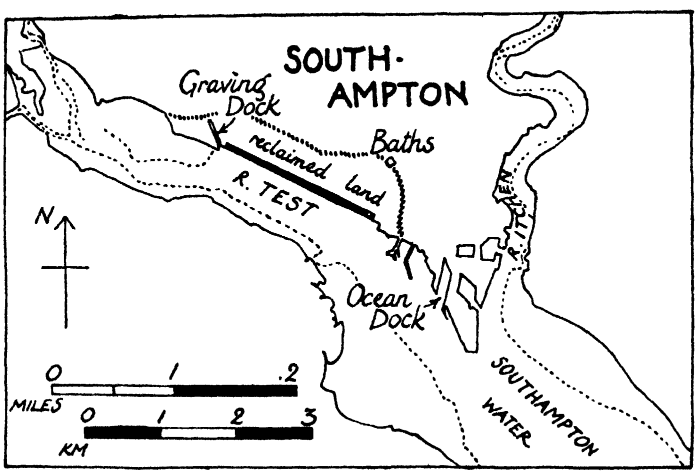

Fig. 1. Sketch map of the submerged estuaries of the rivers Test and Itchen at Southampton, with the three sites referred to in the text, the Graving Dock, Baths and the Ocean Dock.

by Shore & Elwes (1889) from the excavation of the Ocean Dock, and the excavations and borings in the New Dock excavations have been carefully followed and recorded by Prof. Sherrifs and F. W. Anderson. Our section showed three horizons of abundant prostrate trees, the lowest probably pine and the others alder, whilst the upper peat surface below the harbour mud was penetrated thickly with vertical alder roots from some alder-fen since destroyed. At two horizons pockets of shell marl were particularly abundant, making somewhat discontinuous layers. The whole section suggested deposition in a wide river valley filled with fen, which at some

times became dry and at other times shallowly flooded. The borings of the Southern Railway engineers indicated that similar deep deposits occurred in a belt across the region of the reclaimed dock area, and doubtless show the course of the ancient valley of the River Test.

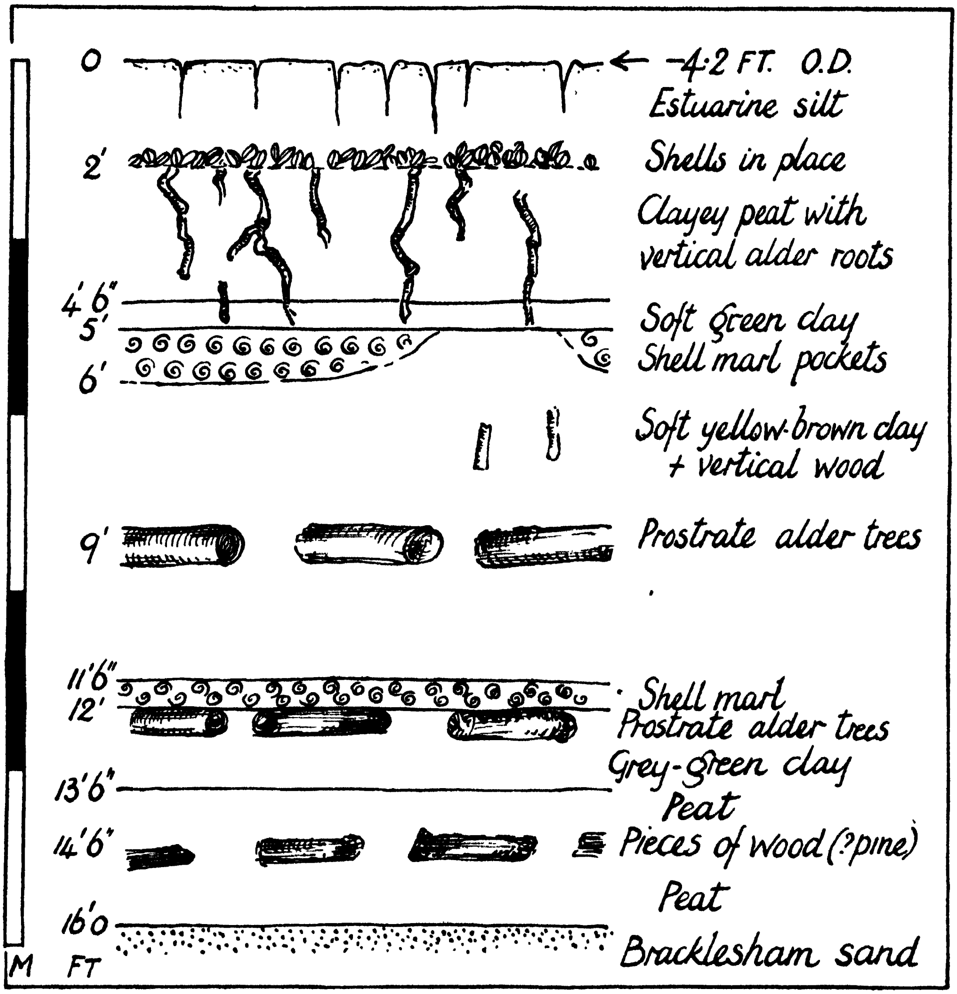

Fig. 2. Section exposed in one part of the excavations for the King George V Graving Dock, Southampton.

The application of pollen analysis to samples from the profiles gave the results shown in Fig. 3: a first attempt to prepare the material by the alkali method gave a diagram with large gaps where samples were too sparse for counting, but supplementary preparations made recently by the Erdtman method allowed these gaps to be filled in: this supplementary work has been done through a grant paid by the kindness of the Department of Scientific and Industrial Research, towards extension of pollen-analysis investigations in the British Isles. The diagram has been divided

into the system of zones already employed for East Anglia (Godwin), Shropshire (Hardy), and Berkshire (Clapham, A. R. & B. N.). The base of the peat falls in the birch-pine zone (IV) which can be considered as the pre-Boreal, a reference supported by the substantial amounts of *Salix* pollen, and pollen of *Myriophyllum alterniflorum*, a most typical component of Swedish as well as British deposits of this period. This lowest sample contains very much pollen of aquatic plants. In zone V pine is dominant and VI is characterized by the rapid establishment of oak and elm,

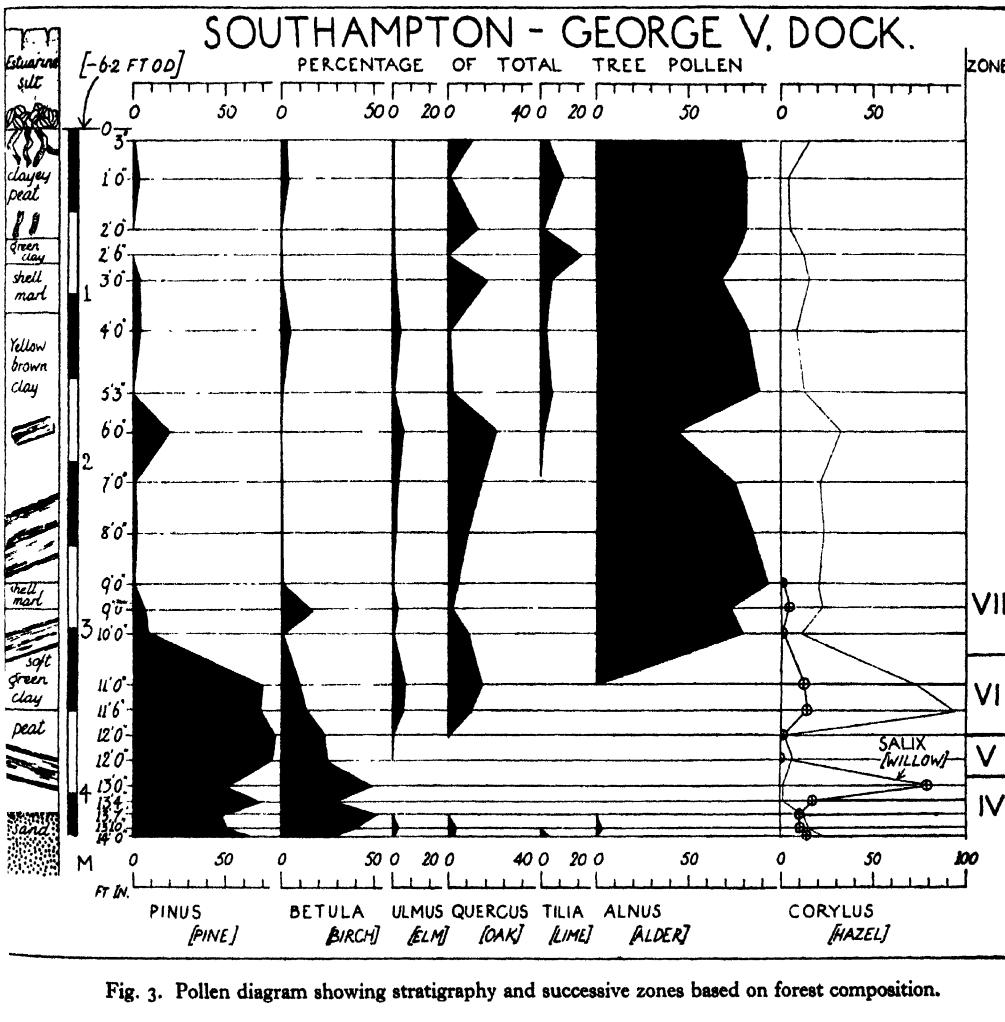

Fig. 3. Pollen diagram showing stratigraphy and successive zones based on forest composition.

with very high hazel values. The opening of zone VII is shown by the expansion of alder at the expense of pine. An alternative interpretation might set this zone as beginning at the 6 ft. o in. level where *Tilia* first appears continuously, but this level is preceded by amounts of *Alnus* which are much larger than those found in zone VI*c* in other parts of England.

Unfortunately the samples do not allow the recognition of clear zones above the top of zone VI, but it is evident that a very long time must have elapsed after the opening of zone VII about *c.* 6500 B.C., before submergence cut short the accumulation of these fresh-water valley deposits at a height somewhere above −6·0 ft. O.D.

It would naturally add much to the value of the observations of these river deposits if it were possible to correlate them clearly with archaeological horizons.

Two discoveries have been made which might possibly serve this purpose. Mr McHaffie showed us a flint flake embedded in a peat sample recovered in 1930 by Mr C. Peel from the base of a deep trench excavated on the west side of the Corporation Baths. The peat bed here was said to lie at about 18 ft. below Ordnance Datum: it was about 3 ft. thick, and very rich in well-preserved remains of oak, beech and hazel trees. The determinations were not checked, and it should be noted that beech has never been shown certainly to have existed in Britain at the period to which we provisionally relate this peat.

The flake had certainly been humanly struck, was unpatinated and showed no secondary flaking: it might have been waste from a flint industry of any period. From the depth at which it occurred the peat around it cannot well be younger than Boreal or Atlantic in age (zones VI–VII), since peat formation in the Test valley must have resulted from general water-logging, and since the transition between zones VI and VII at the Graving Dock lies at about −16 ft. O.D. Analysis of the peat sample in which the flake was actually found shows the following result:

Pinus	5 %	*Tilia*	0 %
Betula	2 %	*Alnus*	87 %
Ulmus	3 %	*Corylus*	17 %
Quercus	6 %		

This is probably either late Boreal or early post-Boreal in age, corresponding well with the zone of doubtful age, from 6 ft. to 10 ft. 6 in. in the main pollen series. Thus referable to either zone VI*c* or early VII, the flint is presumably Mesolithic.

The other artifact was described as found in the Ocean Dock excavation, where deep deposits of peat and shell marl from the Old valley of the Itchen were overlaid by deep estuarine mud (Shore & Elwes, 1889). The object was a round hammerstone with hour-glass perforation, "found near the bottom of the peat twenty feet below the surface of the mud". "This hammerstone when brought to Mr Shore, had adhering to it some of the peaty tufaceous material in a pocket of which it was found." The level of the deep peat below Ordnance Datum is difficult to make out, but it must have been of the same order as that in the Graving Dock and was probably contemporary, especially since the site is so close, and the deposit of such similar character.

The quartzite hammerstone with hour-glass perforation is a type commonly found in a Mesolithic context (for example it is part of the Danish Maglemose cultures: see Clark & Rankine, 1939). It could readily, therefore, have come from Boreal or early Atlantic layers. It is a great pity that no trace can be found of this tool, which may still have upon it the tufaceous marl which Shore describes, and which would still yield material for pollen analysis.

This brief account may be taken to show that deposits covering a large part of the post-Glacial period still lie submerged in Southampton Harbour and ought to be most carefully examined when new excavations expose them again. The possibility of obtaining a clear relation to mesolithic culture is good and it evidently might prove possible to get a closer estimate of the progress of marine transgression than that which the present evidence supplies, viz. that at this site marine transgression affected levels of −6·0 O.D. not before the middle of zone VII of our scheme of forest history.

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A BOREAL TRANSGRESSION OF THE SEA IN SWANSEA BAY

DATA FOR THE STUDY OF POST-GLACIAL HISTORY. VI

By H. GODWIN
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(With 5 figures in the text)

CONTENTS

Introduction

All along the coast of south Wales submerged peat beds occur, some exposed by erosion on the foreshore and others disclosed by the deep dock excavations at ports such as Swansea, Port Talbot and Barry. There has unfortunately been little systematic collection of samples from these beds for examination by recent methods of analysis: although incomplete the present observations do represent some progress in this direction, and give preliminary measurement of some stages of the post-glacial marine transgression.

The samples were obtained through the good offices of Prof. O. T. Jones, F.R.S., during the sinking of bore-holes on behalf of the Swansea Corporation in 1931. The line of bore-holes from which peat samples were obtained extended in a north-easterly direction from the east end of the King's Dock and Queen's Dock which themselves lie to the east of the town of Swansea (see Fig. 1). The line of borings crosses the margin of the great Crymlyn Bog which lies a little further east, separated from the sea only by the sand-dunes of Crymlyn Burrows. Following our receipt of, and preliminary investigation of the peat samples from the bore-holes, we were enabled, through the courtesy of the resident engineer, Mr E. Llewellyn Davies, to have transported to Cambridge a complete vertical monolith of peat 13 ft. 4 in. long, representing the total thickness of the bog deposit in its marginal portion. The position from which this sample came is indicated in Fig. 1. Small uncontaminated specimens taken at intervals from the length of this peat monolith were subjected to pollen analysis, along with the peat samples from the bore-holes.

The technique of preparation was that of hot alkali digestion, followed by oxidation and acid hydrolysis (Godwin, 1934). For counting some of the samples I

employed the services of Mrs H. B. Whitmore, who was paid as my assistant by the kindness of the Department of Scientific and Industrial Research, for other counts I am indebted to my wife.

Deep borings

Samples were sent to Cambridge from each of eight bore-holes designated respectively 31, 32, 33, 34, 35, 36A, 37 and 38. The samples were from peat beds or peaty layers traversed by the bore, and though the exact depth of the bed is given

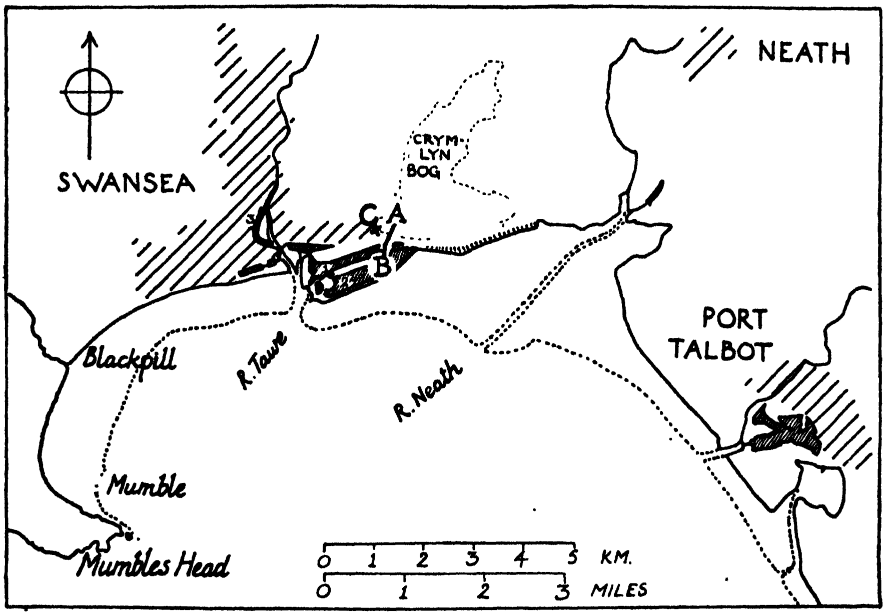


Fig. 1. Sketch-map to show the western side of Swansea Bay with the docks and Crymlyn Bog just to the east of the town of Swansea. The line of bore-holes is shown by the line labelled *A–B*: B.H. 30 is at the north end of it and B.H. 38 at the south. The position of the peat monolith is shown by the cross labelled *C*. The docks are numbered: 1, King's Dock; 2, Queen's Dock; 3, North Dock.

for each sample, it is not known from what part of the bed the sample (about 6 in. in thickness) came. The ground surface was levelled so that all the beds are relatable to Ordnance Datum. The peat beds rest upon, and are separated by, a fine grey silt and clay which we can presume to have been of estuarine origin,[1] and in places this was stated by the boring engineers to have contained peaty matter. The diagram of Fig. 2 is intended to show the exact vertical positions of the samples dealt with, and the lithological class recognized after careful examination. It is of particular importance to note in the two very thick peat beds at the top of bore-holes (B.H.) 30 and 31, that the portion seen represents only one sample from an unspecified depth.

It will be recognized at once that the beds sampled fall into three main groups: (1) an upper set of brown *Sphagnum-Calluna* peats, not extending below −10 ft.

[1] "Estuarine" is used provisionally and for convenience: it may not represent the exact conditions of deposition as judged from the microfauna.

O.D., (2) a lower set of black compacted fissile peats, between −35 and −55 ft. O.D. (mostly between −46 and −55), and (3) an intermediate set of clayey silts between −16 and −40 ft. O.D., all containing some plant remains, but the upper ones paler grey in colour, containing less organic material, and the lower ones of the set much darker in colour and including recognizable layers of compacted black peat.

In addition to these types we may note the peaty sand of the top sample of B.H. 33, at +16 ft. O.D. (this might have come from any wet-dune hollow of very recent date), and the dark grey silty clay of the top sample of B.H. 38, where the broken shell fragments suggest a former beach line in front of the dune ridge.

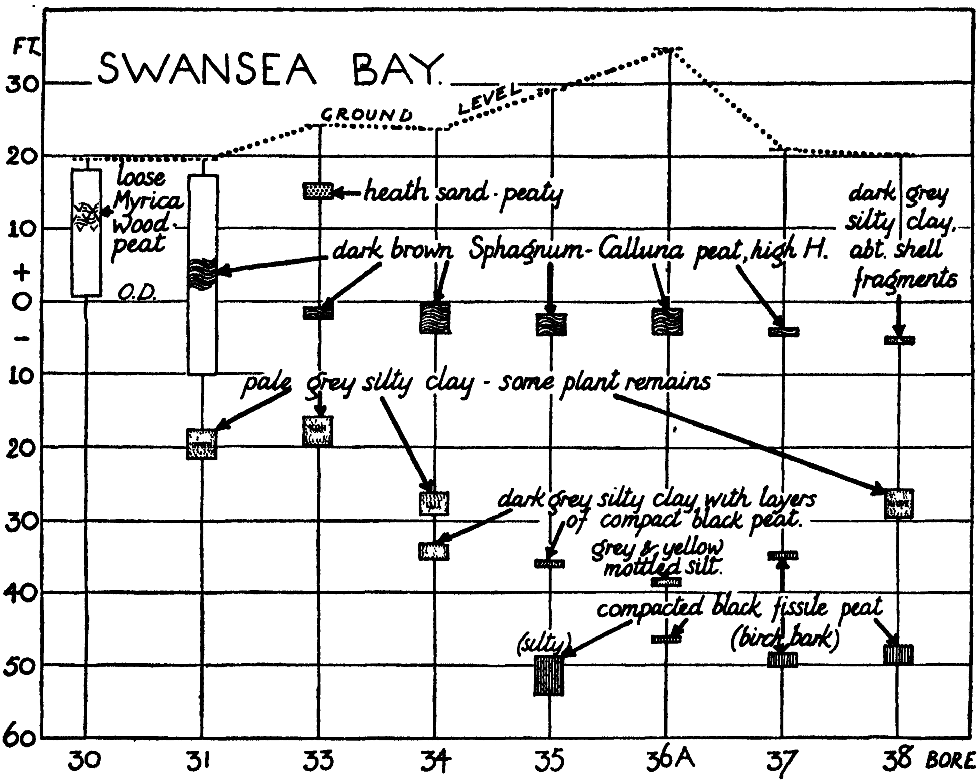


Fig. 2. Diagram to show the lithological character of samples recovered from eight borings made in Swansea Bay in 1931. The vertical lines show the separate bore-holes, and each bed of which a sample was taken is shown by a rectangle at the appropriate depth in relation to Ordnance Datum, and of appropriate thickness.

The diagram gives very clear evidence for a continuous upper peat bed extending at about −1 to −5 ft. O.D., from B.H. 33 to B.H. 37 beneath the thick sand of Crymlyn Burrows, which at B.H. 36A reaches almost +35 ft. O.D. All the samples from this upper peat bed (and also those from the thick upper beds of B.H. 31) were a dark chocolate brown well-humified *Sphagnum-Calluna* peat, such as is quite typical of the "old" peat below the "Grenzhorizont" of so many British raised bogs, e.g. Tregaron Bog, Shapwick Heath, etc. At B.H. 35 it contained layers of less humified

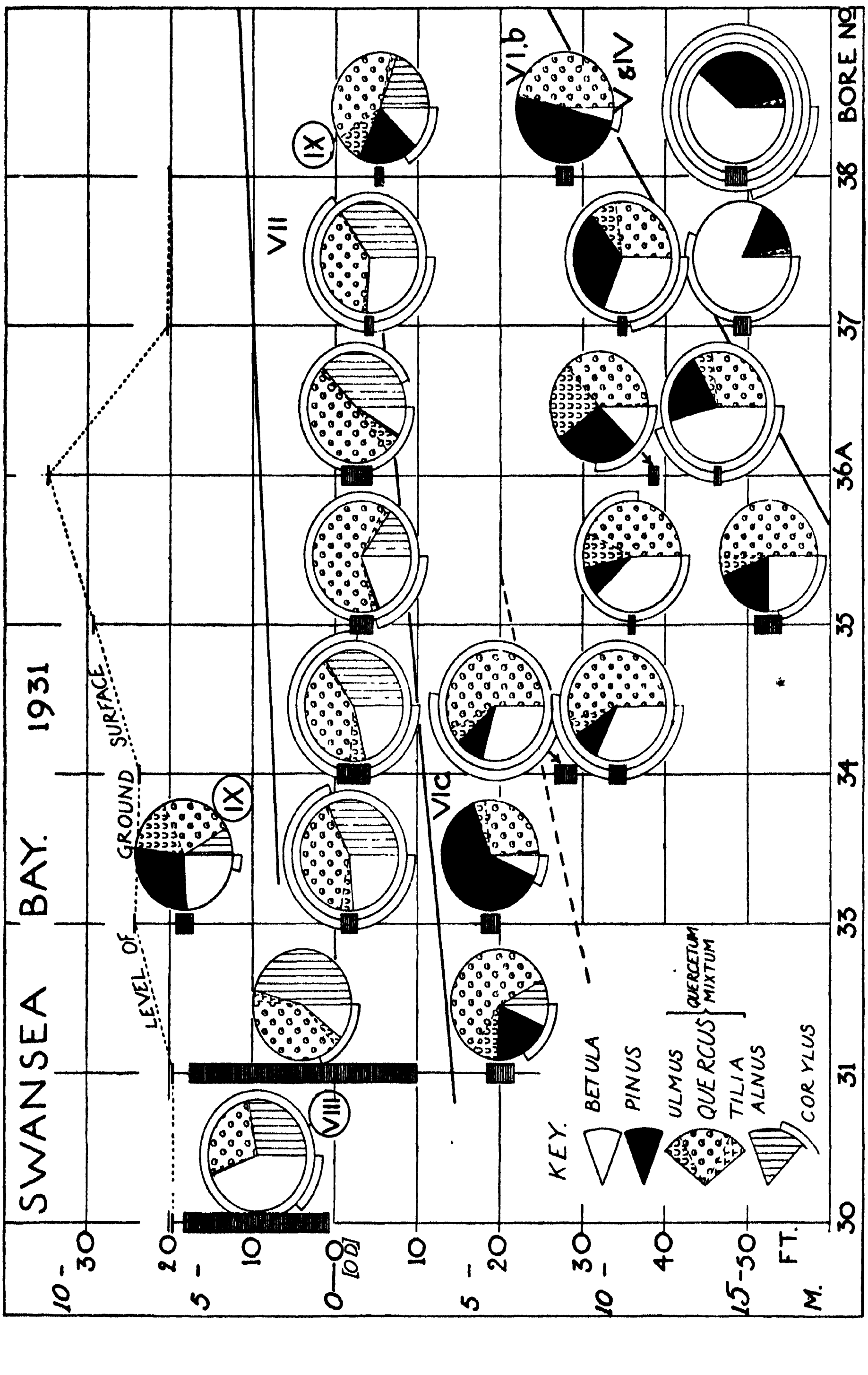
Sphagna, and at B.H. 36A some *Phragmites*. There can be little doubt that this bed is continuous with the base of the still-growing Crymlyn Bog, and may indeed, in view of the compression by so much overlying sand, correspond with a considerable thickness of the peat in the open bog. At B.H. 31 the upper peat descends to − 10 ft. O.D., where, according to the data from adjacent borings, it fills in a former channel. The presence of typical oligotrophic raised-bog peat in such a wide extent over an estuarine silt, suggests a remarkable degree of freedom from the effects of drainage water, or of the sea, and recalls the extension of similar, but less oligotrophic peats over the Fen Clay at similar levels in the East Anglian Fens. The loose peat with leaves and twigs of *Myrica gale*, found in the peat from B.H. 30, is by no means out of accord with the *Sphagnum-Calluna* peat found in the other samples: similar layers with abundant *Myrica* are very well developed in the upper part of many raised bogs, e.g. Borth Bog, Cardiganshire.

The other peat beds, which occur at much greater depths, are of quite different character. They are black or dark grey, fissile compacted peat, often silty and structureless. They contain little recognizable plant material beyond monocotyledonous rootlets, and are probably much humified fen peats. The lowest sample in B.H. 37 contained much yellow birch bark, and the sample next above in the same bore contained the plant fragments typical of a fresh-water lake mud, with two large fruits of *Ceratophyllum demersum*, and several radial plaques which appeared to represent colonies of filamentous algae. These deeper peat samples all have the character of fen peats, which accumulated where drainage water of eutrophic character accumulated on the surface of the estuarine silt at times when interruption of some kind took place in the effects of the general marine transgression.

It might be thought that the middle sample of B.H. 37 really belongs to the group of dark silts which lie in other bores at the same level, being merely exceptionally peaty. From evidence of peat type and of levels it might be supposed that these deeper peats were referable to a single deep peat bed at about − 50 ft. O.D. The pollen analyses make it clear, however, that two at least must be recognized.

The analyses of the peat samples from the bore-holes are represented diagrammatically in Fig. 3, and illustrate a wide range of forest composition readily understandable when considered in the light of long, continuous pollen series such as those from Tregaron, Cardiganshire (Godwin & Mitchell, 1938), Shropshire (Hardy, 1939), and to come to a site less than 20 miles distant, Ffos-ton-Cenglau, Glam. (Hyde, 1940). The samples fall into clearly separated groups, each referable to a distinct period of forest history, and this grouping has been indicated in the diagram, each group receiving an appropriate zone number in accordance with the scheme developed by the author (Godwin, 1940) and already applied by A. R. and B. N. Clapham (1939), Hardy (1939), and Hyde (1940).

The two lowest samples in B.H. 37 and 38 are referable to the birch-pine zone IV, i.e. the pre-Boreal, for *Betula* is strongly dominant, and *Pinus* is the only other tree represented in large amount. The mixed-oak forest trees are barely indicated at all. These features appear to guarantee the age of the bed clearly enough, although hazel pollen is present in remarkably large amount in the sample from B.H. 38, and the



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birch-bark present in bulk in the sample from B.H. 37 suggests a strong local effect has contributed heavily to the high birch pollen percentage. Eight more samples fall into a later subzone, which we can recognize with fair certainty as VI*b*: these are the lowest and middle beds of B.H. 34, 35 and 36A, and the middle beds of B.H. 37 and 38. Zone V and subzone VI*a*, separating these samples from the earlier ones, are not represented. The zone allocation follows decisively from the pollen composition: *Betula* has receded in importance, but still with *Pinus* is present in large amount; on the other hand, *Quercus* is now present in very large proportion, with big percentage of *Ulmus*, but no *Tilia* and no *Alnus*. In subzone VI*a* *Ulmus* would predominate over *Quercus*, and in subzone VI*c* *Alnus* and perhaps *Tilia* would be present in small amount. This general expectation comes from a consideration of many British diagrams, but it is very strongly supported in the nearest diagram, Ffos-ton-Cenglau (Hyde, 1940), in which were also noted these very high elm and oak percentages during zone VI: they much exceed values in this period from any other part of the country, although the forest history has shown a *parallel* drift.

In the absence of several complete local diagrams relating to this period, it is not advisable to attempt a subgrouping of these eight samples. The only sample doubtfully referable to another subzone is the middle sample from B.H. 38 which perhaps belongs to VI*c*: this sample is also different lithologically from the others, agreeing in this respect with the two bottom samples of B.H. 31 and 33. These two come from beds at about −20 ft. O.D. and clearly belong to zone VI*c*, for in comparison with samples already considered, *Betula* is still smaller in amount, *Ulmus* has diminished in importance, small amounts of *Alnus* are present, and *Corylus* is present in much smaller proportions than in VI*b*; in other parts of the country, very high *Pinus* values are not infrequent in this subzone and they are very high in B.H. 33.

Zone VI is taken as the Boreal period: before considering younger samples, two points of special interest may be raised concerning the ten samples we have placed in it. Firstly the two peat beds in B.H. 35, though separated by almost 15 ft. (4·6 m.) of silt are both referable to the subzone VI*b*, thus indicating that rapid submergence was in progress at this period. Similarly between the middle of the highest sample in subzone VI*c* (−19 ft. O.D.) and the middle of the lowest sample in subzone VI*b* (−52 ft. O.D.) there is a distance of 33 ft. (10 m.). This possibly represents a crude net transgression rate of about 5 ft. (1·5 m.) per century. Secondly, the basal peat in B.H. 35 lies some 4 ft. lower in level than the basal peat in B.H. 37 and 38, although it is considerably younger: similarly the basal peat in B.H. 36A is practically at the same level as the much older basal peat in B.H. 37 and 38. This fact would seem to suggest either (1) marine retrogression between the formation of the peats of zone IV and those of zone VI*b*, or (2) rather remarkable spatial isolation of sites B.H. 35 and 36A from B.H. 37 and 38 over a long period, so that the former was fresh water and the latter estuarine. The closeness together of the bore-holes (330 ft. between B.H. 36A and 37) much weakens the second suggestion, and it may be recalled in relation to the first that in the south Baltic region after a marine (*Yoldia*) stage in the pre-Boreal, there was a fresh-water (*Ancylus*)

stage in the Boreal, although marine transgression (*Littorina*) returned later. The detailed records of the deeper parts of the borings made by the engineers are of interest in this connexion. They show that the line of borings crosses an ancient channel in the hard rock extending to about −120 ft. O.D. where it is deepest at B.H. 37 and 38. This old channel is filled with very variable beds of clay, sand, gravel and boulders which form a flat floor at about −50 ft. O.D., but also extend much more thinly up the valley sides. Over these lie the light silty clays and peat beds, which are the record of the more or less interrupted transgression we are now concerned with. The nature of these thick basal deposits must be left to independent geological treatment. The base of the silts and peats in B.H. 38, 37, 36A and 35 rests directly on a bed of clay with stones, and presumably indicates the time when the transgression first flooded a surface which had long been exposed. It seems quite possible that the two older peat beds from B.H. 37 and 38 were really growing on the old valley surface long before the transgression reached this level. It is unfortunate that the present evidence on this point should be so slight, but the facts may serve nevertheless to stimulate further enquiry.

The five samples from just below Ordnance Datum in B.H. 33, 34, 35, 36A and 37 show at once great resemblance to one another and large differences from the deeper beds. *Betula* has fallen to about 20%, *Pinus* is almost entirely absent, and *Alnus* is present in large amount. *Quercus* is still the most abundant tree, but associated with it in the Quercetum mixtum, are both *Tilia* and *Ulmus*, the latter in much smaller amount than in zone VI*b*. It seems certain that such a constitution is indicative of zone VII of post-glacial forest history, although it is not possible to say from what part, and the single sample from an unknown depth in the deep upper peat bed of B.H. 31 probably also belongs to zone VII.

The single small sample from some unknown depth in the thick upper peat bed of B.H. 30 is probably referable to zone VIII, for it differs from the five samples just discussed in the higher *Betula* percentages, and extremely small amounts of *Ulmus* and *Tilia*. The hazel pollen percentage of 113%, may possibly include wrongly determined grains of the *Myrica gale* so strongly represented in the macroscopic remains.

Although the upper peat bed must have suffered compression beneath the coastal dunes, it seems unlikely that it can represent anything like the full thickness of the present Crymlyn Bog peat with which it is continuous. Admitting that the samples examined may have come from unknown depths in the upper peat, nevertheless not one of the five is young enough to fall into zone VIII, which covers at least the last 2000 years. It seems probable that deposition of this part of the upper peat bed ceased before the onset of zone VIII.

The peaty sand sample at +18·0 ft. O.D., in B.H. 34 shows a pollen composition quite different from that of samples at lower levels: *Pinus* and *Ulmus* have returned in large amount, and *Alnus* has diminished together with *Corylus*. These modifications suggest the changes consequent on the planting, felling and drainage operations of the recent historic period, and the sample is referred to zone IX, as indeed accords with its position on dune sand below made ground. The upper sample in

B.H. 38 lies at about the same level of $-5{\cdot}0$ ft. O.D., as the base of the upper peat bed, but its pollen composition is entirely different. It shows instead close similarities with the upper peat sample from B.H. 34 just considered, and as we know it to be a beach deposit it seems reasonable to suppose that modern heath peat from the dune surface was incorporated in it before it was covered by made ground. It also may be referred to zone IX.

It may be worth while in concluding these comments on the pollen samples, to note the remarkable way in which the deductions of age on the basis of pollen composition, agree with the clear data of lithological character and level.[1]

Peat monolith

The constitution of the peat column is shown on the left of Fig. 4. Three layers are recognizable. The uppermost 3 ft. (90 cm.) below the living bog surface is a decayed *Sphagnum-Carex* peat containing very little wood. Below this, to a depth of 7 ft. (2·1 m.), is a peat of rather similar character with frequent small twigs of birch and alder. Below this to the surface of the underlying clay is an alder fen-wood peat with abundant large branches of alder wood, and at 9·0 ft. (2·9 m.) a large branch of oak. There was a hazel nut at 10·0 ft. (3·1 m.) and at 12 ft. 6 in. (3·8 m.) a large sandstone pebble in the peat. It will be seen that the results of pollen analysis both in tree pollen and non-tree pollen reflect this threefold division of the peat bed. The pollen samples were taken at intervals of about 20 cm. and the results are given in Fig. 4. The alder fen-wood below 7 ft. is indicated by the high values for tree-pollen frequency per unit volume of peat, and by high alder-pollen values. Oak is high also as might be expected of a tree growing *in situ*, and other tree-pollen curves are correspondingly depressed. After a transitional zone of fluctuating dominance in which lowered tree-pollen frequencies and higher fern-spore percentages indicate more open conditions, the middle peat layer is reached. In this the tree-pollen frequency, though less than in the lowest layer, suggests some local influence, and the high birch values agree with this. The upper peat layer begins at about 3 ft. (91 cm.), and the relative treelessness of the bog surface at this time is shown by the lowered tree-pollen frequency and the substantial values of ericoid and grass pollen.

Nothing in the peat monolith at all resembled in character the dark, well-humified *Sphagnum-Calluna* peat already described as present in the bore-holes just below sea-level, and indeed the section as a whole suggests a sequence of more eutrophic communities, such as might have been associated with a lagg or river-valley margin of a raised-bog. Only detailed boring would reveal the relation of this series either to Crymlyn Bog or the acid peat below the shore dunes. At the same time the peat rests directly upon a silty clay of presumed estuarine origin, and the thick peat over it indicates a long period of immunity from marine invasion. Since the surface of the peat monolith was at $+19{\cdot}5$ ft. O.D. its base on the silty clay must have been at $+5{\cdot}5$ ft. O.D., some 10 ft. higher than peat bed shown by the

[1] It may also be noted that the foraminiferal content of the series of samples has already been investigated by Dr W. A. Macfadyen, whilst the geology of the site as a whole is being recorded by Prof. O. T. Jones.

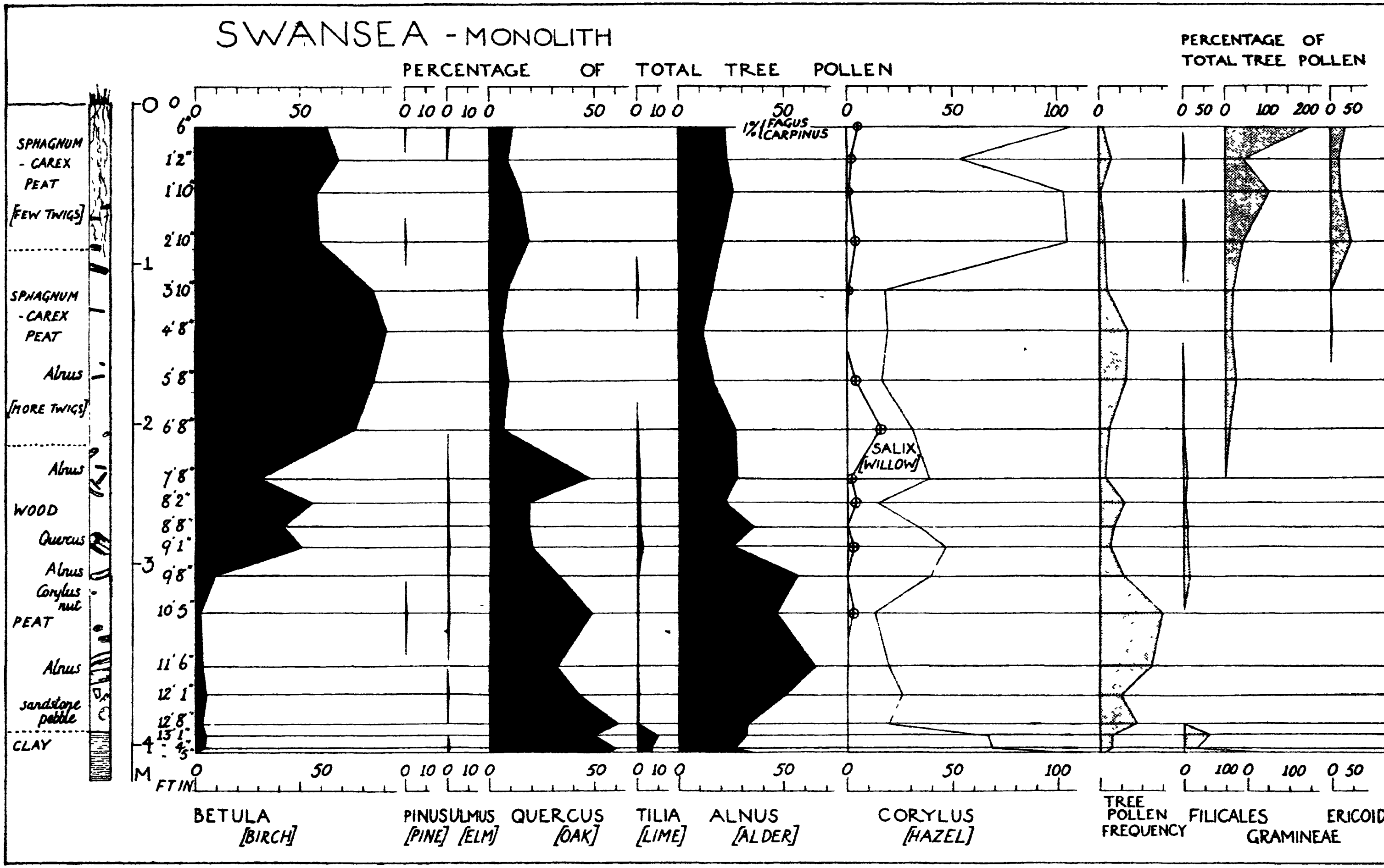

Fig. 4. Pollen analyses from peat monolith taken from drain. On the left, the gross peat stratigraphy, and on the right (half-tone) the non-tree pollen, and the relative density of tree pollen on the different samples all made up by the same alkali digestion technique.

borings below Crymlyn Burrows: the clay below the peat monolith may well be attributable to a later transgression than that below the peat bed under the dunes.

In the face of the pronounced local influences which affect the pollen diagram, it is clear that its interpretation in terms of regional forest history will be difficult. Already, however, in South Wales, Hyde has taken the replacement of *Sphagnum-Betula* wood peat by a *Molinia* peat devoid of wood, to indicate the border or "Grenzhorizont" which indicates the sudden climatic degeneration at the close of the Bronze Age (Fox & Hyde, 1939; Hyde, 1940). This horizon, which separates zones VII and VIII of our scheme, evidently falls at about 7 ft. in the diagram. The behaviour of pollen which is *not* local may be held to support this view, for it is at about this level that the curve of *Tilia* shows the pronounced falling off, before its virtual disappearance, a common and consistent feature at this level in diagrams for the many parts of Britain.

The age which we should ascribe to the base of the pollen series cannot be readily deduced from the pollen curves themselves, but the accumulation of about 6 ft. (1·8 m.) of wood peat before the "Grenzhorizont" suggests the middle or later part of zone VII. It may be noted that nothing at the top of the diagram corresponds with the pollen spectrum of the heathy sand from + 18·0 ft. O.D., in B.H. 34, and there is nothing indicative of the effects of human influence on forest composition. Peat growth may well not have continued here actually up to the present time.

A last point of general interest in regard to the forest-history of the country as a whole is the exceedingly small representation of the pine: it is even lower here than in other Welsh sites during the same period, e.g. Ffos-ton-Cenglau and Tregaron Bog.

SUBMERGED PEATS ALONG THE NEIGHBOURING COAST

The submerged peat beds of the south Wales coast have been recognized at many places and described by numerous observers, particularly in the last few years by T. N. George. In two instances pollen analyses have been made of the submerged peat, and in one instance there is a possibility of an archaeological correlation. Samples appear not to have been recovered hitherto from so great a depth as 50 ft. (see Fig. 5).

It will be instructive to consider briefly what has already been written of the submerged peats in Swansea Bay and then those found successively eastwards. Dr George (1936) has shown that along the whole of the western side of Swansea Bay from Mumbles almost to the mouth of the Tawe there is a peat bed about 4–5 ft. thick, with its surface approximately 15 ft. above Ordnance Datum. It is generally overlaid by sand or made ground, and rests upon blue clay. This bed is definitely too high in level to correspond with the upper peat we have described from B.H. 33–37, although it might well correspond with some part of the long peat profile in the Crymlyn Bog. This view is supported by a short series of pollen analyses carried out by von Post (1933) on the submerged forest bed exposed on the shore at Blackpill. There seems little doubt from the position of this bed on the foreshore and its continuity, that it represents the extensive peat bed described by George from immediately behind the shore. The presence of beech pollen in four

out of the five samples leads von Post to refer the bed to a period as late as the Iron Age, and this reference to zone VIII is supported by the high values for birch pollen. Since beech pollen has not been encountered in the samples examined by us, except at the top of the peat monolith, it is important to note that evidence of charcoal has been produced by Hyde (1937) which shows that beech was native in south Wales in the early Iron Age.

The link between these observations on the west side of Swansea Bay and our own on the north is provided by other observations. First, at a bore-hole close to Brynmill, George describes an upper peat bed 2 ft. thick and about 8 ft. above O.D., which is separated by clay 10 ft. thick from another peat bed which extends from −1 to −5 ft., and itself rests upon clay. The same author (George & Griffiths, 1938) has described, from sites close to the mouth of the river Tawe, a peat bed 1 ft. thick, the base of which is 2 ft. below O.D. covered by clay and resting upon *Scrobicularia* clay. It appears that erosion would have removed the higher peat bed had it ever been present. Very close to the site of these observations lies the Swansea North Dock, the deposits of which were described by Moggeridge (1856). He describes three peat beds separated by *Scrobicularia* clay, but his data do not allow a very accurate relation to Ordnance Datum. If it is assumed that the upper peat lay between +10 and +18 ft. O.D., then the second peat lay between −0·5 and −2·5 ft. O.D., and the lower peat bed lay at −5·5 to −8·5 ft. O.D. These last three sets of observations clearly suggest that hereabouts there are two main peat beds on the shore, the upper at about 10 ft. above Ordnance Datum and the lower just below Ordnance Datum. The upper falls into zone VIII and the lower into zone VII. It is the lower of these two which is represented in our present observations. It may be noted in passing that the upper of these two beds has the more claim to be considered a "submerged forest", for there are abundant trees exposed in it along the foreshore on the west of Swansea Bay, and they were recorded from the upper peat of the North Dock by Moggeridge.

At Port Talbot, some 5 miles to the east, Gibson (Strahan, 1907) described an upper peat bed between −1 and −2 ft. O.D. with *Scrobicularia* clay above and below it, and a bottom peat at −25 to −26 ft. O.D. An impersistent peat bed at about −8 ft. O.D. has been mentioned subsequently, and this falls at about the level of the lower peat described from the North Dock, Swansea. Our own B.H. 34 and 38 showed peaty clay at about −28 ft., which is fairly close to the level of Gibson's lower bed.

A good deal farther to the east we are fortunate in having the carefully recorded sections from the Barry Docks (Strahan, 1896). The most substantial peat bed here disclosed was the uppermost, which lay between −3 and −5 ft. O.D.; it contained willow, pine and oak trees and a fragment of a polished Neolithic flint celt. Taking the evidence of this artefact at face value, it supports the reference of this bed to the end of zone VII, and the supposition that the peat bed at this level is of similar age all along this coast. *Scrobicularia* clay lies above and below this bed and at about −10 ft. O.D. there was an impersistent marshy peat. At −20 ft. O.D. was a persistent third bed with large oak trees. It will be noticed that this is the level of the peaty

clays in B.H. 31 and 33 to which we referred zone VI*c*. Lastly, at −35 ft. O.D. was another peat bed with much wood, from which oak, hazel, dogwood, hawthorn and willow were identified by Clement Reid. This assemblage agrees well with the assumption that the bed corresponds in age to the peats at the same level in our B.H. 34–37 which were placed in zone VI*b*.

The most interesting information about these beds at Cardiff comes from a recent investigation by Hyde (1936). At the East Moors he records a peat bed (or beds) varying from 6 in. to 6 ft. in thickness and lying between 12·5 ft. above Ordnance Datum and 0·5 ft. below it. Hyde gives a detailed pollen diagram through the peat bed where it is 2 ft. 6 in. thick (76 cm.), and its surface lies at +8·3 ft. O.D. Wood was present in the sector, and it seems likely that local factors have influenced the pollen diagram heavily: the sequence of maxima from alder, oak to birch is one fairly often encountered in the development of wood peat immediately over salt-marsh clay. *Tilia* pollen is present in such very large amounts that there must have been a very prolific local source for it. It seems possible that the analyses agree with a reference to the later part of zone VII, and the early part of zone VIII, although this would make the bed somewhat earlier than the uppermost of the peat beds of Swansea Bay. We may lastly mention some unpublished observations from Combwich near the mouth of the river Parret on the south side of the Bristol Channel, and south of Cardiff. Here, below 14 ft. of pale silt, was a peat at +7 ft. O.D. containing abundant trees of *Alnus* and *Taxus*. Below it was 7 ft. of clay and then at Ordnance Datum a further peat bed about 0·7 ft. in thickness. It seems not improbable that these two beds again reflect the double character of the upper peat beds about Ordnance Datum already emphasized in Swansea Bay. The Combwich upper peat bed shows low birch pollen, but rather high elm and lime, and in relation to unpublished series from the Somerset Moors these features suggest reference to a zone not later than VII.

The most obvious relationships of these peat beds have been summarized in Fig. 5. Taken together there is a fairly strong suggestion of peat beds at no fewer than eight or nine different levels and that beds at the same level, after zone V, are of the same age.[1] Apart from the bed represented by the lower peat in B.H. 37 and 38 it seems clear that these successive peat beds represent stages of halt or slight retrogression in the progress of a considerable marine transgression, the greater part of which was accomplished during the second half of the Boreal period, but which showed continued but smaller movement in later periods. How far this orderly sequence of peat beds separated by silt and clays can be taken to reflect the changing balance between eustatic and isostatic factors affecting relative position of land and sea level, we can hardly say at present, but there is much evidence in Scandinavia, where the isostatic recovery was very great, that periods of equilibrium or reversal of movement were quite frequent.

[1] The varying tidal range along the coast does not seem to have had any disturbing effect on these correlations, but it should be noted that this possibility remains, especially for the easterly sites at Cardiff and Combwich, where the pollen zonation is least well established. It might well be that the submerged peat bed at Westbury-on-Severn, near Gloucester, which lies at about 14 ft. above O.D. (Prevost *et al.*, 1901) corresponds in age with beds lower in level in the region now considered.

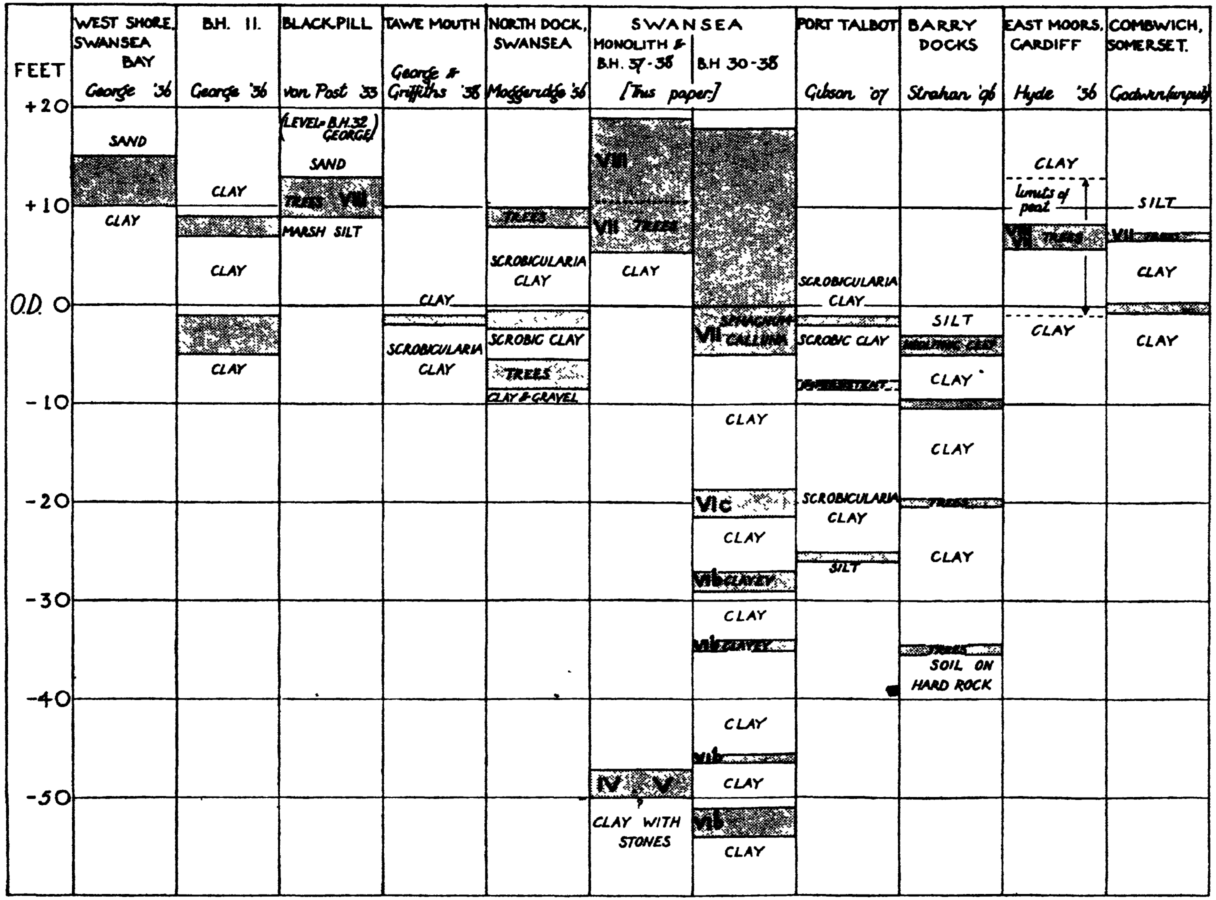

Fig. 5. Diagram to show the disposition of coastal peat beds at important sites in Swansea Bay and eastwards along the Bristol Channel. The peat-beds are shown in half-tone, and where pollen analyses give a clue as to age the appropriate zone number appears in Roman figures. The table gives reason to believe that beds of similar level occur at many places on this coast line, and reflect widespread conditions of peat formation between periods of marine transgression. Note especially the tendency for there to be one peat bed about +8 or +10 ft. O.D., and another just below Ordnance Datum.

Summary

Borings in Swansea Bay, south Wales, have shown the existence of a series of peat beds separated by silty clays, the lowest peat bed lying more than 50 ft. below sea-level. Pollen analyses show that most of this series was formed in the middle and second part of the Boreal period, during which time the net rate of marine transgression must have been very great. There is some indication that here as in Scandinavia this rapid transgression possibly followed a period of marine retrogression in earlier pre-Boreal times. The subsequent long time-span is represented by peat and clay beds higher than – 20 ft. O.D. A peat bed constantly present just below Ordnance Datum was typical *Sphagnum-Calluna* peat and appeared part of the deep Crymlyn Bog here lying just behind the coastal sand-dunes. A pollen-sequence through the long monolith from the edge of this bog showed it referable to zones VII and VIII of the author's pollen diagram zonation.

An account is given of the records already made from important sites along Swansea Bay and to the east, where coastal peats have been encountered. It appears that these beds occur over a wide distance at approximately the same levels and show a similar character. When available, pollen-analyses and human artefacts support the view that after zone V beds at the same level on this limited stretch of coast are of the same age, and referable to the same stage of halt or slight inversion of the general marine transgression. Eight or nine such levels seem to be indicated at various times from the pre-Boreal onwards.

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THE WATERMARK DISEASE OF WILLOWS

I. HOST-PARASITE RELATIONSHIPS

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(With Plate 5 and 2 figures in the text)

1. Introduction

The watermark disease of willows is chiefly of importance because it attacks *Salix caerulea* Sm., the cricket-bat willow, causing considerable financial loss to growers. A brown or black stain, accompanied by waterlogging, develops in the wood; this discoloration renders bats made from diseased wood unsaleable. Also such bats have a tendency to splinter. A diseased tree can usually be recognized in summer because the leaves on certain twigs turn bright red in colour; sometimes whole branches are so affected. This symptom is known as "red leaf". The affected branches die back and ultimately a "stag's head" is formed. Numerous adventitious shoots arise from the diseased stem and a new crown may be formed.

From the academic viewpoint, watermark disease is the only recorded bacterial disease of trees in which the pathogen (*Bacterium salicis* Day; Dowson, 1937) is entirely confined to the vessels of the wood. This greatly facilitates the investigation of the pathological effects produced in the host.

The work recorded here corroborates and greatly extends the original description of the disease given by Day (1924). For the most part the facts described relate to diseased *Salix caerulea*, but much wood of other diseased willows (particularly *S. alba* and *S. fragilis*) has been examined, and except in minor details the disease behaves in the same way in each host plant.

2. The watermark

If a recently infected two-year-old branch is cut in summer the whole of the central region of the wood is waterlogged and stained. A close examination shows that this stained area is made up of three distinct regions (Text-fig. 1). In the outer part of the first year's wood is a narrow zone which is stained black; this zone extends as an irregular ring right around the annual ring. The area between this black ring and the initial parenchyma is stained red-brown and is waterlogged. The whole of the wood internal to the black ring, including the pith, is sodden with water and is stained a dark brownish-green colour. The wood of the outermost ring is usually unstained except on its innermost boundary.

On exposure to the air the cut surface turns first red-brown (almost scarlet) and then black. The black stain is not localized owing to the copious exudate of sap which spreads over the whole of the cut surface.

When a thin section is cut from a diseased branch the colours are immediately changed; the black-stained ring appears brown in colour and the rest of the stained wood is light brown. There is thus no real black stain in the wood, and as the liquid squeezed from the wood is not coloured the dark colours must be due to an optical effect, more of the incident light being absorbed in the central waterlogged region than in the drier wood on the outside.

Fig 1.

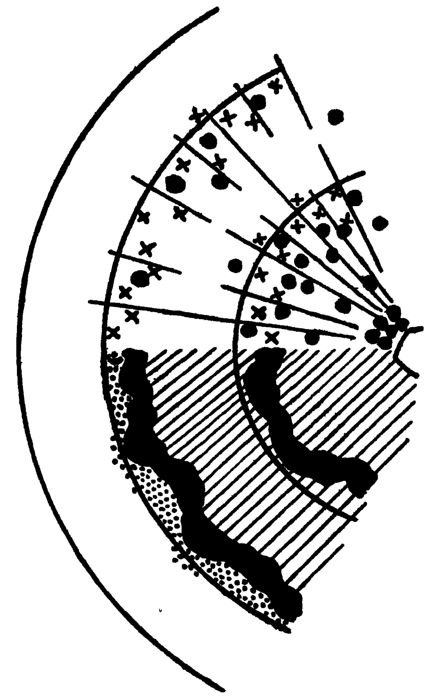

Fig. 2.

Text-fig. 1. Two-year-old twig, diseased for one year. Cross-section. Diagrammatic. Below—the stains: dots, wood watersoaked and red-brown; hatching, wood waterlogged and brown-green; black, black stain. Above—the distribution of occluded vessels: crosses, vessels occluded with bacteria in the first invasion; circles, vessels occluded with bacteria in the second invasion. The black lines represent lengths of brown-stained rays.

Text-fig. 2. Three-year-old branch, diseased for two years. Cross-section, diagrammatic. Below—the stains. Above—the distribution of occluded vessels. Symbols as in Text-fig. 1.

The watermark seen when a branch is cut may take other forms, depending upon the age of the branch cut, upon the length of time during which the disease has been present in the branch, and also upon the conditions under which the tree has been grown. In branches which are more than three inches in diameter the ring of dark stain may be replaced by a circle of discrete patches, or the stain may be present as an arc of a circle. In branches diseased for more than one year there may be two or more dark rings (Text-fig. 2) or a discoloured centre with a dark ring on the outside.

3. THE ORGANISMS ASSOCIATED WITH THE DISEASE

In the early stages of the investigation it was noticed that during the winter few colonies of *B. salicis* appeared on isolation plates, whereas certain other organisms were almost constantly present. During the summer, although *B. salicis* was then easily isolated, the same organisms appeared. Accordingly their distribution in the wood and their relationship to the disease were investigated.

Bacteria were isolated from the wood in two ways. The following method was used for all-the-year-round isolations from single annual rings. The bark of the branch was removed and the wood surface sterilized with alcohol. The branch was then cut across with a sterile razor and the wood whittled away until the required annual ring was exposed, care being taken to keep the required wood free from contact with the razor. Fresh surfaces were then cut and thin sections of the required wood dropped into sterile water. After half an hour the diffusion was streaked out on heart-infusion agar plates.

By this method of isolation the bacteria from all the cells of the wood are obtained on the agar plates; it obviously cannot be used in a study of the distribution of the bacterial species within the annual ring. In order to do this, thick sections were cut with a sterile razor and mounted on a smear of agar on a sterile cover-slip in the damp dissecting chamber of a Janse Péterfi micromanipulator. The bacterial contents of individual vessels were removed by the aid of micropipettes operated on the micromanipulator. The contents of each pipette were ejected into a drop of sterile water on an agar plate; this was subsequently smeared evenly over the surface of the agar.

On isolation plates made by the above methods it is seldom possible to obtain a pure stand of *B. salicis*, other bacteria being associated with the pathogen in the wood. These bacteria are of three kinds. One, a white organism (*bacterium* A), is nearly always associated with the pathogen in diseased wood. It gives the characteristic reactions of *B. aerogenes*. The second, a yellow organism (*bacterium* B), is usually but not constantly present. Colonies of other yellow organisms may be present in any single isolation, but only *bacterium* B has been isolated with any degree of regularity. The third is a green-fluorescent organism (*bacterium* C); it has been repeatedly isolated, particularly from long-diseased wood, but other (casual) green-fluorescent organisms may be present in any one isolation. These three organisms have been isolated repeatedly from diseased *Salix caerulea* and *S. alba*; they have also been isolated from *S. fragilis*, *S. cinerea*, and *S. caprea*.

The organisms were recognized initially by the colony form, by the growth on potato, and by the behaviour in dextrose, sucrose, maltose and lactose solutions. Later, ten strains of each organism from *S. caerulea*, five of each from *S. alba*, and the strains from the other three willows, were compared in more detail. The individual strains of one organism often differ slightly in their cultural characters and fermentation reactions, but the differences between strains isolated from different host species are no greater than the differences between strains isolated from the

same host species. All the strains of one organism are here considered to belong to the same species.

The isolation experiments have shown that the relative proportions in which colonies of the organisms A, B, and C, and of *B. salicis*, are obtained on any isolation plate vary with the time for which the wood has been diseased, and with the time of the year. The results are set out in Table 1. In compiling this table, an isolation was made from diseased wood and the presence or absence of each of the four bacteria noted. This was repeated several times (never less than five times, usually more than ten times) using wood from a different source for each isolation. In order to facilitate comparison, the final results have been "scaled" so that each of the figures in the last four columns represents the number of times that the particular organism was present in ten isolations. Isolations were made summer and winter from diseased annual rings of different ages. In addition, an attempt has been made to indicate on an arbitrary scale the proportions in which the bacteria appear on any average isolation plate.

Table 1 shows that the bacterial population of any annual ring changes with time, the proportion of viable *B. salicis* rising to a maximum and then falling to zero, whereas the proportion of the other organisms steadily increases. This fact, when taken in conjunction with the histological data put forward below, suggests that the bacterial types A, B and C are probably secondary organisms. This is confirmed by the fact that inoculations, using pure and mixed cultures of the organisms, did not reproduce the disease in healthy trees.

Table 1. *Occurrence of bacterial types in watermarked* Salix caerulea

Age of annual ring	Age of branch	Season of isolation	*Bacterium salicis*		*bacterium* A		*bacterium* B		*bacterium* C	
1	1	Winter	2	I	10	II	0		0	
1	2	Winter	2·5	I	10	II	1·7	I	0	
1	3	Winter	1·4	I	10	II	1·4	I	0	
2	2	Summer	10	III	10	II	4	I	2	I
2	3	Summer	10	II	10	II	5	I	2·2	I
2	2	Winter	3	I	10	III	4	I	6	I
2	3	Winter	1·7	I	10	III	5·8	II	5·8	I
3	3	Summer	1·3	I	10	III	8·7	II	8	II
3	3	Winter	0		10	III	10	II	10	II

Number of colonies per plate: I = 0–10; II = 10–50; III = over 50.

The importance of these organisms, which are present from an early stage of the disease, must not be underrated; the study of the development of the pathological symptoms and of the death of the tree has shown that there is no reason to suppose that the secondary bacteria do not play as important a part as *B. salicis*. As these bacteria occur in the same situations as *B. salicis* their secretions and metabolic products have the same chance of affecting the living cells of the wood as have those of *B. salicis*, and it has seldom been possible to separate the pathological effects produced by one organism from the effects of the bacterial complex. To continue to

ignore the effects of secondary organisms in the study of a disease, as has often happened in the past, cannot be justified. The present work has not, however, elucidated the problem of why *B. salicis* can cause the disease whereas other organisms, apparently equally well fitted to live in the environment of the vessels, cannot. It is possible that *B. salicis* has simpler nutritional requirements and that its presence in the vessels creates the environment needed by the other bacteria.

Descriptions of the organisms

Bacterium salicis Day. This organism has been isolated from *Salix caerulea*, *S. alba*, *S. fragilis*, *S. Russelliana* and *S. cinerea*. Strains from the first four hosts were compared in detail; no important differences were noted and as these four strains differed in minor points only from the strain described by Dowson (1937), no further description is given. Dowson & Callan (1937) found that the *alba* strain of the organism would infect *S. caerulea*, and *vice versa*. This result has been confirmed; it has also been found that the *fragilis* strain will infect both *S. caerulea* and *S. alba*, but it has not been possible to infect *S. fragilis* with any strain of *B. salicis*.

bacterium A. Rods, $1 \cdot 75 \times 0 \cdot 8 \mu$, motile by 2–5 peritrichous flagella. White growth on heart-infusion agar. White growth on autoclaved potato, usually with a grey discoloration of the potato. Acid and gas from dextrose, sucrose, maltose, lactose, salicin and glycerol solutions. Ammonia produced, nitrates reduced, gelatin not liquefied, no indol produced, V.P. positive, methyl red negative, grows in citrate solution.

bacterium B. Short rods, $1 \cdot 4 \times 0 \cdot 75 \mu$, motile by 3–7 peritrichous flagella. Yellow growth on heart-infusion agar; yellow slimy growth on autoclaved potato, the potato is usually not discoloured. Acid from dextrose (gas), sucrose, maltose, salicin and glycerol. Ammonia produced, nitrates reduced, gelatin liquefied, no indol produced, V.P. positive, methyl red positive.

bacterium C. Elongated rods, $2 \cdot 25 \times 0 \cdot 8 \mu$, motile by 1–3 polar flagella. White growth on heart-infusion agar with green diffusible pigment. Brown slimy growth on potato, the substrate being liquefied and a black discoloration produced. Acid from dextrose and glycerol, good growth without acid in sucrose, maltose, lactose and salicin. No ammonia produced, nitrates not reduced, gelatin liquefied, no indol formed, V.P. negative.

4. PATHOLOGICAL HISTOLOGY

The multiplication of the bacteria leads to the blocking of the vessel; the bacterial plugs so formed may occupy a considerable length of the vessel. The bacteria in the plugs lose their motility and later in the year become impregnated with a brown staining-substance, thereby losing their viability. Isolations from diseased wood, made with the micropipette, showed that the bacteria form a mixed population in the vessels. Gas is formed in many of the vessels containing bacteria; the gas-filled vessels are present in wood cut under water and kept under water until examined. The gas is probably formed during the fermentation of the reducing sugars in the sap.

Tyloses are formed into the vessels and may be so numerous as to form a pseudo-parenchymatous tissue. If the vessel contains bacteria these are compressed into compact masses between the tyloses. Gum of the type associated with the attacks of many fungi on woody tissues (e.g. with the attack of *Stereum purpureum* on plum trees—Brooks & Moore, 1923) is only rarely found in the vessels. In transverse sections the vessels frequently appear to contain brown gum-like contents, but this is because many of the tyloses contain large quantities of oil which is stained brown.

There is no delignification of the walls of the infected elements but, as described below, the middle lamella of the wall may be dissolved away. In the very last stages of the disease, when the wood is permeated with secondary bacteria, there is often a change in the composition of the cell walls which gives them abnormal staining properties but even at this stage of the disease there is no solution of the walls.

The bacteria do not directly attack parenchymatous ray cells and it is only after the death of these cells and the disintegration of the contents that the bacteria establish themselves in the rays. Ray cells in the neighbourhood of infected vessels usually contain large amounts of oil, and are impregnated with a red-brown staining substance. During the first stages of the disease the ray cells are the only cells in the wood which are discoloured.

The "typical" distribution of bacteria in diseased branches was studied throughout the year; the facts presented for each age of branch are based on an examination of up to two thousand sections and it is thought that the generalizations made are accurate. To facilitate presentation of the data, the pathological histology as seen in January is described; changes throughout the year are referred to below.

(*a*) *One-year-old twig on a diseased branch*

Such a twig appears to be free from all pathological symptoms but a careful examination of sections reveals that thin layers of bacteria line the walls of many of the vessels in the outermost wood. The rest of the wood is free from infection.

(*b*) *Two-year-old branch, diseased for one year*

The pathological symptoms in such a branch are shown diagrammatically in Text-fig. 1. In the inner annual ring, up to 50 % of the vessels in the outermost wood may be occluded with bacteria. In many of these vessels the bacteria are impregnated with a brown stain; micropipette isolations show that these bacteria are non-viable. In other vessels the bacteria are not stained and are viable; they consist of a mixture of bacteria A and B, occasionally with *B. salicis*. Occasionally the fibres adjacent to an occluded vessel are permeated with bacteria. The proportion of vessels which are occluded varies around the annual ring; the occluded vessels are not confined to the outer wood although they are most numerous there.

In the outer part of the annual ring the walls of all the ray cells are stained brown, as are the contents of many of the cells. The length of ray so affected varies greatly; in the inner wood the ray cells are usually normal and unstained.

In the outermost infected region, small cracks in the wood connect vessel with vessel, or isolated vessels with infected ray cells. These cracks are formed by the

splitting of the wood along the middle lamellae of the walls. They take a diagonal or tangential path through the wood and may provide an extensive system of communication between infected elements. The cracks are usually filled with bacteria; their formation and their significance are discussed below.

In the outer annual ring the cells of the initial parenchyma (Metcalfe, 1939), and also the first-formed cells in most of the wood rays, are stained brown. Throughout the rest of this annual ring the ray cells have normal protoplasmic contents and contain enormous numbers of very minute starch grains. Usually the vessels in the earliest-formed wood, particularly those abutting on the initial parenchyma cells, have very distorted walls; many are occluded with bacteria (Pl. 5, fig. 2). The middle wood seems at a glance to be free from infection but close examination shows the presence of a very thin layer of bacteria around the walls of many of the vessels. In the outer wood these layers are more pronounced; occasional vessels may be occluded.

(c) *Three-year-old branch, diseased for two years*

The pathological symptoms in such a branch are shown diagrammatically in Text-fig. 2. In the inner annual ring all the ray cells are stained brown. Most of the vessels in the outermost wood are occluded with masses of brown-stained bacteria (non-viable); the brown stain may have diffused from the vessels and the ray cells and stained the wood elements in the neighbourhood. A large proportion (5–20 %) of the vessels in this ring are occluded with viable secondary bacteria; these vessels are evenly distributed throughout the annual ring, although the vessels of the leaf-trace bundle and the vessels on the inner edge of the wood are usually occluded (Pl. 5, fig. 3). Such vessels occluded with secondary bacteria provide evidence of a second bacterial invasion of the wood. Later still, all the tissues in this annual ring become permeated with secondary bacteria; very many bacterial types are present at this stage.

In the middle annual ring many vessels on the innermost edge are occluded with brown-stained, non-viable bacteria, many with viable secondary bacteria. The vessels in the middle wood of the annual ring are usually free from infection; in the outer wood the pathological symptoms resemble those described above for the oldest ring in a two-year-old branch. In the middle annual ring the rays are usually brown stained, but if the ring is wide only the inner and outer ends of the rays may be so stained.

In the outer annual ring the distribution of bacteria and the pathological effects are almost identical with those described above for the corresponding annual ring.

(d) *Older branches*

In five- and six-year-old branches, which had been diseased for one year, the dark stain was confined to the inner wood of the previous year's annual ring; vessels occluded with bacteria are most numerous in this region. The outer wood of this annual ring and the outer annual ring are unstained and are free from bacterial infection. In older branches, diseased for more than one year, the inner diseased

annual rings are permeated with viable secondary bacteria, and vessels occluded with these bacteria are evenly distributed.

(e) *Root wood*

In root wood the distribution of the diseased vessels is similar to that in shoots. The whole of the centre of the root appears to be waterlogged and near the junction of the annual rings a dark stain is present in the wood. This dark stain may not extend completely around the annual ring; even in two-year-old shoots it may be present as an arc of a circle. In older roots, diseased for more than one year, two such zones of darkly stained wood may be present as in the shoot. In winter, in the younger roots, the bacteria-filled vessels are confined to the dark-stained areas. Tylosis formation occurs freely in diseased root wood and a very high proportion of the vessels of the inner annual rings may be occluded with a pseudo-tissue derived from tyloses. All the tissues of the root may be permeated with secondary bacteria which form a thin layer around the walls of most of the vessels.

The fact that there are secondary bacteria in all the vessels of diseased roots is of great importance; in the spring the water absorbed from the soil has to pass through these vessels and the bacteria will be carried into the conducting elements of the stem.

5. The invasion of the wood

There is little information as to how the disease spreads from one tree to another (Day, 1924; Callan, 1939). Once a branch becomes diseased, however, the disease may persist in it from year to year, successive outer annual rings being invaded until ultimately the branch succumbs. It is certain that this infection of an outer annual ring takes place from the inner diseased annual ring and not from some external source. This point is discussed below, as are the processes by which establishment and spread of the disease in this wood take place.

If no infection of the new wood occurs, succeeding annual rings are free from infection, the diseased part is buried as the branch thickens, and the branch recovers from the disease.

(a) *Infection of the new wood*

In late spring the vessels on the inner edge of the new wood may be infected with bacteria, but the bacteria do not normally spread further into the new wood. The outer elements of the new annual ring become infected in late summer. Histological studies have not revealed how this radial spread of infection takes place, but it is certain that the bacteria do not spread along the wood rays.

The inoculation of the healthy wood can occur by way of the emergence holes and boring holes of various insect larvae. All through the summer, until the end of August, copious exudations of sap containing motile bacteria (among them *B. salicis*) pour from such emergence holes. It has been found that the damaged vessels in the new wood become infected. Further observations on this point were made in an experiment in which nine heavily diseased branches were planted as setts. In the following summer one of the setts showed copious bacterial exudations

from numerous insect emergence holes; this sett died the same year, the new annual ring being heavily infected. The other setts showed no exudations, the new annual rings remained uninfected, and all ultimately recovered from the disease.

(*b*) *The establishment of the disease*

A comparison of the data given in § 4 (*a*) and (*b*), will show the total changes which occur in the wood during the first year of disease; a comparison of (*b*) and (*c*) gives the same information and corresponding information for the second year of disease. Knowing the "typical" distribution of bacteria during each month of the year, it is possible to trace the changes which take place in a diseased annual ring in the course of a year.

The infection of the outermost (healthy) annual ring takes place towards the end of the growing season, for the last-formed elements are amongst those infected. The bacteria (which are not motile) spread by diffusion or by passive water carriage in the outer vessels; that they spread more extensively in these outer vessels than in the inner vessels may be accounted for by the fact that the outer vessels supply the leaves on the most distal parts of the shoots (Metcalfe, 1939) and these distal leaves appear to retain their normal metabolism and transpiration stream (and therefore their salt supply) for a longer period than other leaves.

As a result of this slow spread, by the end of the winter most of the outermost vessels contain bacteria. Following the hydrolysis of starch in March and the consequent appearance of high concentrations of food materials in the sap, the bacteria become actively motile and multiply rapidly. They probably spread very rapidly at this period, both upwards and downwards, under their own locomotion but much of the apparent spread is due to the rapid multiplication of the bacteria in the thin films around the vessel walls. So rapid is the establishment of the bacteria in spring that within a month a new "disease zone" has been formed throughout the tree, both above and below ground; the vessels are occluded and oil accumulates in the ray cells which become impregnated with brown stain.

During the summer the bacteria spread locally in the outer part of the annual ring by means of the small "cracks" described below. During the late summer and autumn the bacteria in the vessels become impregnated with the brown stain; it is probable that this impregnation inactivates them, for as mentioned above it is difficult to isolate *B. salicis* from diseased wood in winter.

From May onwards there is a progressive re-invasion of the wood of the innermost diseased annual ring by secondary bacteria; all parts of the wood are affected by this invasion, which may be very extensive. The secondary bacteria probably invade the aerial parts from the roots. The waterlogging of the central parts of the wood results in many vessels which would otherwise be empty being filled with water; the secondary organisms can spread from the roots into the aerial parts along these vessels. If the upper parts of the branch show red-leaf, and start to die back, the re-invasion of the diseased wood is rapid as it is taking place from above as well as from below. At this stage the fungus *Cytospora chrysosperma* gains an entry into the wood and spreads rapidly as the branch dies.

Thus early in the summer a two-year-old branch which has been diseased for one year begins to assume the appearance typically shown in January by a branch which has been diseased for two years. At the end of August this transition is almost complete. At this time, however, the current year's wood appears to be free from bacteria; only in the autumn is it noticeable that the outermost vessels are infected with a thin wall-layer of bacteria.

(c) *Discussion*

Although motile bacteria are present in the wood until the end of August, inoculation experiments suggest that they do not spread very actively during the summer. Inoculations made monthly between October and March resulted in infected trees, others made between May and September gave no infection. That the greatest invasive activity should be confined to the period following starch hydrolysis cannot be due to the abundant sugar supply alone, for abundant organic materials are present in the sap all through the summer. Probably some inorganic constituent of the sap is important; this is also suggested by the fact that the bacteria associated with the first invasion are confined to those vessels which form the path of the transpiration stream supplying the young shoots in spring. (When the buds open in spring the young shoots reach a length of one or two inches; growth then stops for about two weeks and does not start again until cambial activity has spread right down the tree and a ring of new vessels has been differentiated. It has been established (Metcalfe, 1939, p. 157) that during this period the young shoots are supplied with water through the vessels on the outer part of the last annual ring in the one and two-year-old branches and through the vessels on the inner edge of the last annual ring in older branches.) These are the only vessels to receive a continuous supply of inorganic salts at this period and it is in these vessels that bacterial infection is greatest. When the new annual ring differentiates it largely takes over the function of conduction, and the supply of salts to the older annual ring (which thenceforward reach it by diffusion only) may not be sufficient to support rapid bacterial growth.

The new annual ring usually remains free from infection during the summer; if during the early summer it should become infected, the bacteria multiply and spread very rapidly. This suggests that, given an adequate salt supply, the conditions in the wood in summer are still favourable for rapid bacterial multiplication. The writer suggests that, in view of the relatively high phosphorus requirements of bacteria, the phosphates are probably the most important constituent of the transpiration stream.

6. The cracks in diseased wood

The small cracks formed by the solution of the middle lamellae of the walls are found only in the "disease zone", in the neighbourhood of elements infected with bacteria. It has been established that they are present in the wood before sectioning.

Although in most lignified tissues the pectic material of the middle lamella has undergone chemical change, in healthy willow wood pectic substances are present

in the middle lamella, as they can be dissolved away by boiling with ammonium oxalate, by prolonged boiling with water, or by pectinase preparation, leaving cracks between the elements similar to the natural cracks in water-marked wood.

Commercial pectin was purified by repeated refluxing with alcohol, and the dried sterile powder added to tubes of a sterile solution of inorganic salts. The tubes were inoculated with the four species of bacteria; only in the tube containing *bacterium* C did growth appear, the other tubes (including the controls) remaining free from growth and glucose. Thus *bacterium* C is able to utilize pectin and is probably the cause of the cracks in the wood. The bacteria gain access to the middle lamella by way of the pit membranes; not infrequently these are completely dissolved away.

During their spread along the middle lamellae of the cell walls (Pl. 5, fig. 4) the bacteria come into contact with uninfected vessels and ray cells, and there is no doubt that these are colonized. The cracks thus mark the path of the tangential spread of the bacteria within the annual ring. This path can often be followed for some distance in the wood, from ray to ray, from vessel to vessel. Owing to the relationship of branch to stem (Metcalfe, 1939) this tangential spread within an annual ring enables the bacteria to gain entry into the wood connected with branches whose point of insertion is above or below that of the original infected branch.

Summary

1. In addition to *B. salicis* Day, three other bacteria have constantly been isolated from watermarked willow wood. The bacterial population of diseased wood changes with time; *B. salicis* is inactivated after the first year and there is a second invasion of the wood by the three associated organisms, leading to the complete permeation of all the tissues by bacteria.

2. The distribution of bacteria in the wood and the pathological effects are described in detail.

3. The infection of healthy wood, and the spread of the bacteria in such wood, are discussed in relation to the persistence of the disease in the tree from year to year.

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EXPLANATION OF PLATE 5

Fig. 1. End of vessel element occluded with bacteria. × 1000.

Fig. 2. The "disease zone" at the junction of two annual rings, showing occluded vessels. × 400.

Fig. 3. Leaf-trace bundle, the vessels of which have been occluded during the second invasion of the wood. × 400.

Fig. 4. Early stages in the formation of a crack. The bacteria from the vessel are dissolving away the middle lamellae of the fibre walls. × 800.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

METCALFE—THE WATERMARK DISEASE OF WILLOWS

REVIEWS

A Manual of the Liverworts of West Virginia. By N. AMMONS. $5\frac{3}{4}\times 9$ in. 164 pp., 26 pl. + 1 map in text. Notre Dame, Indiana: University Press. Reprinted from *The American Midland Naturalist*, **23**, 3–164, 1940. Price $1.75 post paid.

In recent years some of the most critical and constructive work on the taxonomy of the hepaticae has come from America, but as yet there is no completed hepatic flora of North America as a whole. Attempts have been made to fill the gap by handbooks for individual states. Of these the work under review is an excellent example.

The introductory sections of the book deal with the general features of West Virginia, the general characteristics of liverworts, the collection and preservation of material, etc. There are descriptions of all the orders, families, genera and species, with well constructed keys. The descriptions of the species are concise, but clear, and are accompanied by data on habitats, distribution in the state and in North America in general and useful notes on variation and differences from similar species. All the species are illustrated in the plates and, though the drawings are not of high quality, they are, nevertheless, useful. The classification is that recently proposed by Evans (*Bot. Rev.* **5**, 49–96, 1939) and embodied in Buch, Evans and Verdoorn's "Preliminary Check List of the Hepaticae of Europe and America (north of Mexico)" (*Ann. Bryol.* **10**, 3–8, 1937).

Since West Virginia was "formerly covered by an unbroken forest" and is crossed by the Appalachian mountain system, it is not surprising to learn that it abounds in habitats favourable to liverworts. The composition of the flora resembles that of the western parts of the British Isles in that it is a mixture of north temperate species with southern and tropical elements.

This book may be described as an intelligent compilation and as such will doubtless be useful to workers in the south-eastern United States. Though nearly 70 % of the species are British, it is not likely to be of much value to British hepaticologists because it is rather a straightforward practical manual than a critical work.

P. W. RICHARDS

Plant Physiology. By B. S. MEYER and D. B. ANDERSON. 9×6 in. Pp. x + 696, with 151 figures in the text. London: Chapman and Hall. 1940. Price 24*s*.

This is a general text-book which its authors believe to be suitable for use with any introductory course. It is planned on well-recognized lines, but is rather more detailed than others with which it challenges comparison. It is comprehensive and includes treatment of some of the physico-chemical background to physiology, with chapters on solutions, colloidal systems, diffusion, osmosis and so forth. The water relations of the plant and allied subjects, such as permeability and absorption, receive particularly satisfactory treatment. Metabolism is more briefly and less happily dealt with, and the chapter on the mechanism of respiration is, in some ways, unfortunate. The scheme put forward shows the accumulation of a molecule of glycerol for every molecule of sugar respired. Selected bibliographies, mostly in English, are attached to each chapter together with questions for discussion.

W. O. JAMES.

Introduction to Carbohydrate Biochemistry. By D. J. BELL. $7\times 4\frac{3}{4}$ in. Pp. viii + 112. London: University Tutorial Press. 1940. Price 3*s*. 6*d*.

Carbohydrate metabolism has become the object of intense and rapidly advancing studies, which gain special interest from the degree of co-ordination which is now being achieved between higher and lower organisms and between animals and plants. A general summary of this field is therefore opportune and likely to be valuable to students working in one or other of its corners. The very modest size of this book brings it well within the reach of any serious student, but has imposed a very drastic degree of condensation and omission; and a considerable general knowledge of organic and biochemistry has also had to be assumed. Probably the seven pages devoted to photosynthesis could have been more usefully given to the main purposes of the book. Advanced botany students, specializing in plant metabolism, will find this up-to-date summary invaluable, condensing as it does much collateral information which is important for them, but which is not commonly found in botanical libraries. The numerous formulae are a valuable feature, but contain a few startling misprints.

W. O. JAMES.

SOCIETY FOR EXPERIMENTAL BIOLOGY

THE Society for Experimental Biology usually holds three Conferences a year, at two of which botanical papers are presented. Members may obtain the *New Phytologist* at special rates. Particulars of the Society can be obtained from the Hon. Secretary, Prof. T. A. Bennet-Clark, University College, Nottingham.

FORTY-EIGHTH CONFERENCE, OXFORD, APRIL 1940

Prof. Peters took the Chair at a Review Symposium on Cell Structure. Work on mitochondria was reviewed by Mr G. Bourne, and the Golgi complex by Mr J. R. Baker. Prof. Bernal described certain relevant physical properties of tactoids (anisotropic droplets) and suggested that accumulation of "foreign bodies" across the equator of a tactoid resembled that of the chromosomes at metaphase. The separation of chromatids might be due to the formation of negative tactoids between the centromeres. Mr White reviewed cytological work on the nucleic acids and their behaviour during nuclear division.

Prof. Osborn took the Chair at a session of papers mostly dealing with photosynthesis. Dr Wohl gave an account of the photosynthetic mechanism of purple bacteria. According to French, the curve relating rate of photosynthesis to quanta absorbed is sigmoid. The curve can be explained quantitatively if it is assumed that the photo-process consists of four successive photo-reactions, that the intermediates of the photo-process are unstable, and that the dark process requires an enzyme which is blocked by the dark process to some extent at high rates of assimilation. In green plants, the photo-intermediates seem to be stable and the enzyme seems not to be blocked. If this difference is taken into consideration, the number of carbon-dioxide molecules reduced per pigment molecule per second is found to be of the same order of magnitude in purple bacteria and green plants. This suggests that a "photosynthetic unit" exists also in purple bacteria.

Dr Lehmann described work on the chlorophyll/iron ratio in plants. The ratio of numbers of mols chlorophyll/equivalents of iron varies from about 1 to 5 in general. In *Sambucus*, this ratio increased from about 1 to 4 during the season, but changes in chlorophyll-content could not be directly correlated with gain or loss of iron, although loss of iron precedes yellowing of the leaves.

Dr Scarisbrick gave an account of the reaction $4Fe''' + 2H_2O = 4Fe'' + 4H' + O_2$ which occurs when isolated chloroplasts in contact with a ferric oxalate, citrate or tartrate solution at *p*H 7·9 are illuminated. The effects of light intensity, narcotics, reactivity or other iron salts were referred to. The role of such a process in oxygen evolution during photosynthesis could not be commented on at this stage.

Dr Bishop described work on the flora of beer wort which he termed a study in biochemical ecology. He showed samples of the flora of normal wort (mostly round yeasts), of wort deficient in nitrogenous substances (mostly ellipsoidal yeasts), and bios-deficient wort (bacteria predominating). Four bioses, I (=ino-

sitol), II*a* (=pantothenic acid), II*b*, and a "fourth factor" are required by many yeasts. Other yeasts require vitamins B_1 and B_6, and yet others can synthesize bios II*a* if provided with alanine.

Dr James and Dr Maskell took the Chair during the two halves of the session of papers on carbohydrate metabolism.

Dr C. S. Hanes described his recent work on the enzyme synthesis of starch. The reactions starch + phosphate $\rightleftharpoons$ glucose-1-phosphate $\rightarrow$ mixed hexose-6-phosphates are catalysed by phosphorylase and phosphoglucomutases. When the latter are eliminated the primary reversible reaction: starch + phosphate $\rightleftharpoons$ glucose-1-phosphate could be studied in isolation. Starch synthesized from pure glucose-1-phosphate is very similar to, but slightly less water-soluble than, potato starch. It is completely hydrolysed by β-amylase. The course of synthesis of starch from Cori ester as shown by iodine coloration was demonstrated. In the discussion Dr W. T. Astbury showed X-ray photographs of the synthetic and natural starches. Although similar, they were not identical.

Dr R. J. Allen described the conversion of hexose-diphosphate to triosephosphate by the zymohexase present in pea meal and potato extracts. This conversion is obscured by the activity of phosphatases to some extent. The percentage conversion to triosephosphate is increased at higher temperature and also with increasing dilution of the reaction mixture. In the discussion Dr Maskell suggested that this was to be expected since the reaction is of the type $A \rightleftharpoons 2B$, where $(A)/(B)^2 = k$.

A group of papers from the Oxford Department of Botany by Dr James and his collaborators Messrs Bunting and Heard, Mrs James and Miss Cragg, dealt with glycolysis and oxidation in barley. Data were given which showed that, both in living tissues and dead preparations, fructofuranosides (especially sucrose) appear to be broken down more rapidly than pyranoses or their anhydrides. The presence of hexosediphosphates in young barley tissues may be inferred from analyses of their acid-soluble phosphorus; and hexose diphosphate incubated with extracted barley saps is rapidly broken down. In the presence of sodium fluoride two products are found, inorganic phosphate and an ester highly resistant to hydrolysis, probably phosphoglycerate. The formation of this ester is inhibited by traces of copper, which also irreversibly oxidizes ascorbic acid. Pyruvic acid accumulates in young roots and leaves poisoned with acetaldehyde or certain aromatic sulphonic acids. It has been isolated and identified as the 2, 4-dinitrophenylhydrazone. In digests of barley saps with 1-naphthol-2-sulphonic acid, pyruvic acid has been obtained from hexosediphosphate and from lactic acid. The latter used ascorbic acid + air as H accepting system. A highly cyanide-sensitive ascorbic acid oxidase exists in barley tissues and is the only direct oxidase yet identified; catechol oxidase is absent and cytochrome oxidase doubtful. The system ascorbic acid + air + barley sap dehydrogenates α-hydroxy acids but not the C_4 acids malic and succinic, nor a number of other substances tried. It does, however, appear to oxidize triosephosphate, possibly via an α-hydroxy acid. A working hypothesis of barley respiration, based on these and other data obtained, involves phosphorylation of

hexoses to hexosediphosphate; splitting to triosephosphate; oxidation to phosphoglycerate with removal of H_2 via the ascorbic acid system to atmospheric oxygen; dephosphorylation of phosphoglycerate to pyruvic acid; and decarboxylation to acetaldehyde + CO_2. In young tissues the acetaldehyde may be consumed mainly in synthesis.

Dr Boswell, discussing the oxidizing systems in potato tuber, pointed out that the catechol oxidase system is concerned with only part of the respiratory activity. The present study aimed at determining whether the residue is due to activity of ascorbic or dihydroxymaleic acid direct oxidases.

The addition of ascorbic and dihydroxymaleic acids to slices of potato tuber resulted in a temporary increase in the O_2 uptake. The CO_2 output during the oxidation of dihydroxymaleic acid was in excess of the air line value, while, on the addition of ascorbic acid, the production of CO_2 was initially inhibited and only subsequently accelerated, the net result was a slight increase in the CO_2 output. The curves relating amount of substrate added to O_2 uptake suggested that neither of these substances was oxidized by a direct oxidase system.

The use of malachite green as an inhibitor of dehydrogenases showed quite clearly that ascorbic acid was oxidized by an indirect oxidase system involving a dehydrogenase. This confirms the conclusion of Zilva and Johnson, who, on quite different evidence, stated that no direct ascorbic acid oxidase existed in the potato tuber. The oxidation of dihydroxymaleic acid was not inhibited by malachite green; therefore either a direct oxidase or a peroxidase-H_2O_2 system was involved. That a peroxidase-H_2O_2 system was concerned was shown by the addition of the dihydromaleic acid to potato slices after the addition of excess of the naturally occurring catechol compound when the oxidation occurred with a smaller oxygen uptake but the same CO_2 output as when the acid was added to the tissue alone. It is clear, therefore, that no direct oxidase system involving ascorbic acid or dihydroxymaleic acids is present in the potato tuber, and that the respiratory activity not involving the catechol oxidase system involves some, as yet, unidentified system.

Prof. Bennet-Clark and Miss Bexon discussed the bearing of certain findings regarding respiration of tissue slices on hypotheses regarding water relations of plant tissues. They pointed out that the respiration of beetroot slices is rapidly increased by immersion in their own expressed juice to a rate about double that attained when immersed in most salt or sugar solutions investigated. Fractionation of the juice showed that the active material appeared to be malate and citrate. These, when applied even at a concentration of 1/500 of the concentration in the sap, produced a 100% rise in rate of respiration.

This was held to indicate that the reserve of malate and citrate in the tissue is cut off from the respiratory system, as might be the case if it was enclosed in a vacuole with relatively impermeable membrane. It was suggested that this amounted to evidence of continuous diffusion of malate and citrate across such a boundary which would possibly be accompanied by electro-osmotic effects. This interpretation was not accepted in discussion by Dr Steward, who suggested that the protoplast could accumulate malate and citrate to an unknown concentration from the outside.

WATER RELATIONS OF PLANT CELLS. II

By T. A. BENNET-CLARK AND D. BEXON
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(With 6 figures in the text)

INTRODUCTION

A CRITICAL examination of the classical theory of the water relations of plant cells seems desirable in view of a number of recent developments. The accepted classical theory to which we refer is the view, based largely on the work of de Vries, that the turgid plant cell consists of a stretched wall with contents the hydrostatic pressure of which is due to and equal to the osmotic pressure difference between the solution in the vacuole and that outside the cell.

On this view the protoplast is regarded as an ideal semipermeable membrane, and the cell is treated as an osmotic system in equilibrium. In actual fact, as is well known, the protoplast is relatively readily permeated by a number of electrolytes and non-electrolytes as well as by water. Consequently, if one is to be assisted in interpreting the water relations of the living cell by comparison with any non-living system, one should choose a diffusion system in which the membrane is permeated by solutes and solvent.

The behaviour of such systems has been discussed by Schreinemakers (1938). Certain special features of their behaviour have been known since the time of Dutrochet and Graham as anomalous osmosis, but even now the molecular mechanism of these effects is not completely understood and surprisingly few exact quantitative data are available.

Anomalous osmosis may consist of a diffusion of water from a solution of high osmotic pressure to one of lower osmotic pressure, or it may consist of a diffusion in the "normal" or congruent direction or a generation of pressure greater or less than that to be expected from the thermodynamic osmotic pressure of the solutions. These two types of water diffusion have usually been referred to as negative and anomalous positive osmosis. It may be noted here that Schreinemakers uses the terms negative and positive osmosis in a different sense and prefers the terms incongruous and congruous diffusion for the "negative and positive" osmosis of many writers.

Though the exact mechanism of these diffusion effects is not completely understood, it is known that they are invariably associated with the congruous diffusion of a second component of the system. It has in the past been assumed that potential differences across the membrane associated with this diffusion cause the anomalous diffusion of water which has been likened to electro-osmosis. The diffusing ions must also carry with them atmospheres or shells of water molecules and the possibility that the anomalously transported water is carried by diffusion in this way

should be remembered. If the diffusion of the congruently diffusing component of the system is maintained constant, the associated anomalous osmosis is also constant.

The metabolic processes of living organisms provide the possibilities of continuous diffusion systems. It is easy to construct a non-living model in which a constant hydrostatic pressure is maintained inside a membrane of suitable permeability by constant diffusion of a solute through it, and this pressure is quite unconnected with, and may be greater than, the osmotic pressure of the solution.

The choice of a non-living model consisting of a solution separated from the water phase by an ideal semipermeable membrane as a representation of the living vacuolated cell seems to us, in view of the evidence brought forward in this paper and in a number of other recent investigations (Bennet-Clark *et al.* 1936; Buhmann, 1935; Mason & Phillis, 1939; Phillis & Mason, 1937; Roberts & Styles, 1939), to be unjustified. There seems to be little doubt that the hydrostatic pressure inside a fully turgid cell is often much in excess of the osmotic pressure of the vacuolar sap; moreover, many of the solutes which on the classic theory are supposed to be responsible for the static osmotic pressure are undoubtedly able to permeate the protoplast (cf. the data of de Vries (1884), quoted by Stiles (1924)).

The treatment of the water relations of the cell which we attempt here is made more complete by the development of methods for estimating the hydrostatic pressures of cell contents which are independent of any theory as to their causation. This treatment has been further assisted by the development by Mason & Phillis of methods for the expression of vacuolar sap. Our results confirm some of their conclusions and provide further evidence that their claim that vacuolar sap is expressed under defined conditions is fully justified.

A preliminary account of some of the results discussed in this paper has already been given (1939).

EFFECTS OF SLOW INCREASE OF PRESSURE ON TISSUES

Experimental methods. Pressure was applied to tissue in the course of this study by means of the Buckton testing machine of the Engineering Department, for the use of which and for much helpful discussion we wish to thank Prof. C. H. Bulleid. The testing machine is in effect a balance and weights are applied to the tissue. The maximum weight which could be applied was 50 tons measurable with an accuracy of $\pm 0{\cdot}01$ ton.

The tissue to be pressed was placed between two flat steel plates. Tin foil was placed between the tissue and the surfaces of the steel plates in order to avoid risk of reaction between juice and metal. Weight was applied and could be measured with considerable accuracy as indicated above, but the resulting pressures were not nearly so accurately obtained. This was due to the difficulty of measuring the area on which this weight is actually applied. If the weight is not evenly applied, the actual pressure is greater than the calculated pressure on part of the tissue and smaller in another part.

The effects of pressure on leaf tissues only are dealt with in this paper. Various treatments were applied to them: single leaves and also piles of leaves were pressed; in certain cases piles of leaves were wrapped in cloth before placing between the steel plates. In all except some early preliminary experiments not dealt with here the more prominent veins were not included in the pieces of leaf which were pressed. It was found that cloth wrapping (except when very fine bolting silk was used) caused damage to the tissue by the pressing of threads into it: similar injury was caused by the larger veins when a pile of leaves containing such veins was pressed. Leaves which are somewhat crinkly also receive similar injury when pressed either singly or in piles. These results all confirm the statements of Mason & Phillis who point out the difficulty of pressing leaf tissues in such a way as to prevent shearing. We also find, as they do, that it is essential to construct the pile of pieces of leaf, which are to be pressed, with the greatest care. Carelessly constructed piles start to slip, and the leaves are squeezed out sideways with marked shearing of the tissues at much lower pressures than those which are withstood by carefully constructed piles.

Since it is the epidermis of the leaves which holds the tissue together and prevents squeezing out and shearing of the tissue, it follows that slices of bulky tissues form even less favourable material for examination by this method. Smooth leaves having considerable areas free from prominent veins thus form the most suitable experimental material. The following leaves have been used: *Beta*, *Gossypium*, *Hedera*, *Fagus*, *Parthenocissus*, and *Prunus laurocerasus*. In all except *Gossypium* and *Prunus* varieties used had vacuolar saps containing anthocyanin pigments.

The pile of leaves is trimmed off with a safety razor blade so that all the pieces are the same shape and their area and fresh weight are determined. The edges of the leaves are exposed when the pile is placed between the plates in the press, and on raising the pressure juice exudes from these exposed surfaces and is collected by pipettes with fine drawn-out points. As a rule piles were constructed so as to have an area of about 25 $cm.^2$ and a fresh weight of about 10 g. The procedure followed in raising the pressure is this: pressure is raised to about 5 atm. and the cut edges of the pile are examined for traces of exuded juice. There has been no exudation at so low a pressure as this in any live tissue examined by us. Pressure is raised to about 10 atm. and kept at this until any exudation ceases. It may take 10–30 min. for all the juice to exude; naturally the rate of exudation is greatest immediately the pressure is raised and decreases roughly logarithmically towards zero. Increments of pressure of about 5 atm. are applied after all exudation at any given pressure has ceased, but after a large part of the expressible sap has been removed larger increments up to about 20–25 atm. are applied. The quantities of juice at each pressure were determined. The osmotic pressures of the different samples were determined micro-cryoscopically, the methods of correction of freezing-point discussed by Harris & Gortner (1914) being used. Residual leaf material was examined microscopically and the effect of killing it was investigated. It will be convenient to deal with the six species used separately, and results of a few typical experiments are considered in detail.

Fagus sylvatica, Exp. XIII. All the material was taken from one copper beech tree, the leaves of which contained anthocyan in the vacuoles of epidermal and mesophyll cells. Two piles cut from halves of these leaves were placed side by side in the press. The weight and area of the pile pressed were 11·5 g. and 21·3 cm.[2] respectively. Results are given in Fig. 1 and Table 1.

In Fig. 1 (and in subsequent figures) abscissae of the points show the pressures applied normal to the leaf surface and ordinates show the total quantities of juice exuded up to that pressure; these quantities are expressed as a percentage of the fresh weight before pressing. The lower part of the curve is extrapolated backwards

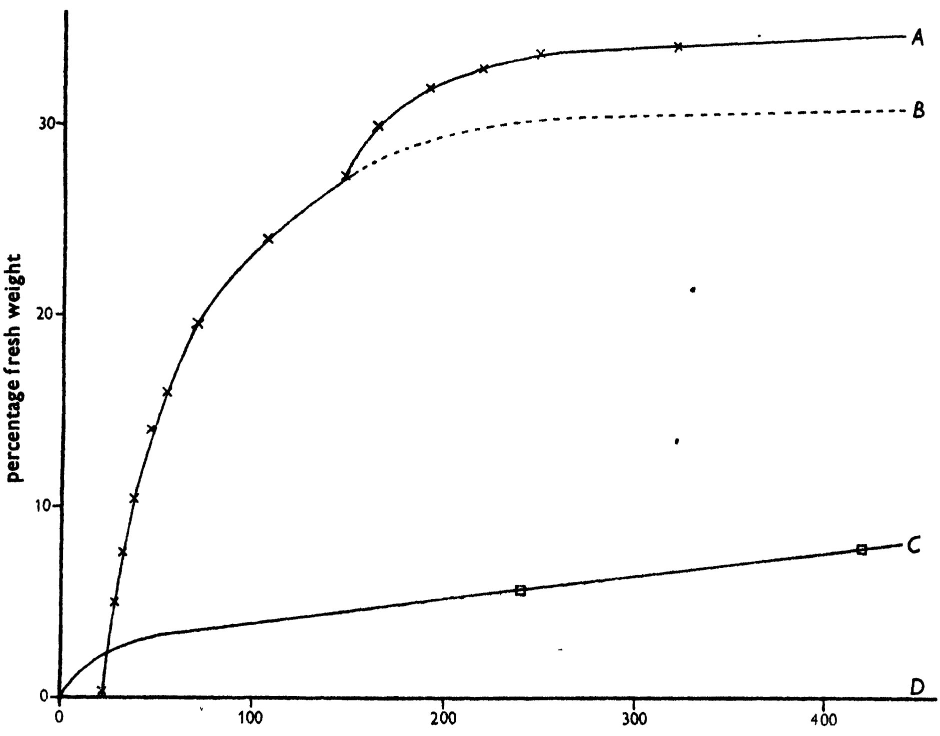

Fig. 1. Volumes of juice expressed by small pressure increments from leaves of *Fagus sylvatica*, Exp. XIII; living leaves —×—; killed residue from fully pressed living leaves —□—.

and cuts the base line at about 21 atm.; at pressures below this no juice is expressed. Above this, successive equal increments of pressure produce exudation of gradually decreasing extra quantities of juice. The curve does not approach an asymptote regularly: it appears to be about to approach an asymptote the ordinate of which presumably is the percentage of juice expressible from the live tissue. Before this asymptote is reached a "break" in the curve occurs, and equal increments of pressure produce at first larger but gradually decreasing extra volumes of exuding juice so that the curve approaches a new asymptote at a higher level.

All the samples of juice obtained before the "break" in the curve were colourless although the vacuoles contained anthocyan. Samples of juice obtained after

the "break" were strongly coloured with anthocyan. Table 1 shows that the former samples of juice had a relatively low osmotic pressure while the latter were high and successive samples had higher values. Increase of pressure above about 1000 atm. produced practically no further exudation of juice from the tissue. Superficially the leaves appeared to be very little injured, and almost no tearing or shearing was observed externally. Sections, however, showed that a considerable number of cells were injured.

The compressed residue of the pile of leaves was then treated with solid carbon dioxide, which killed any cells remaining alive, and was replaced in the press. On raising the pressure a further quantity of strongly coloured juice of high osmotic pressure was obtained as indicated by the lower curve in Fig. 1.

It should be pointed out that the shape of this curve is not established by this experiment in which an insufficient number of separate collections was made. The total quantity of "dead" juice was accurately determined, and the general shape of the curve is known from other experiments some of which will be dealt with later.

The osmotic pressures of the various samples of juice are recorded in Table 1. The anthocyan-free samples have a low value and they appear to consist largely of water from which a great part of the solutes of the cell has been filtered off by the live and relatively semi-permeable protoplast. Anthocyan-free samples of juice had osmotic pressures of 3–5 atm. in certain cases. The solutes responsible have evidently not been set free as a result of breakage or death of certain cells, as such injury would also liberate anthocyan. The exact significance of these rather high osmotic values is not yet quite clear. The anthocyan of the juice obtained under relatively high pressures was possibly liberated as a result of injury which became more extensive as the deformation of the tissues by pressure increased. The increasing osmotic pressures of successive samples confirms this view. Killing by freezing has the effect of enabling more water and solute to be expressed from the press-cake by quite low pressures as indicated in Fig. 1. In our view the quantity of juice represented by the quantity BD is expressible from the live uninjured tissue, and an extra quantity $AB+CD$ is expressible after the injury or death. Rather clearer evidence of the correctness of this view is obtained from other experimental material.

Microscopic observation of sections cut from leaves pressed to the stage just before the "break" in the curve showed that the vacuoles had been reduced to a very small percentage of their original volume, and these residua of the vacuoles were coloured darkly by anthocyanin. The juice obtained from the live uninjured tissue contains most of the vacuolar water. It may also contain cytoplasmic water. After killing or injuring the residue more water can be expressed, and this extra water is derived almost entirely from the cytoplasm since the vacuoles have been reduced in volume at least 90% by the first pressing. The cytoplasmic water washes out the vacuolar solutes, and possibly cytoplasmic constituents, liberated on killing the cell, and a juice of relatively high osmotic pressure and anthocyan content is obtained.

No juice could be collected from the tissue at pressures lower than 22 atm. The extrapolation of the volume-pressure curve downwards to zero volume suggests that the first traces of exudation might have been expected at 21 atm. The beech leaf, like most of the leaves examined by us, only contracts in volume by about 2–3 % in a plasmolysing solution. The consequence of this small degree of turgor stretching is that an application of a pressure lower than the hydrostatic pressure of the contents of the fully turgid tissue expresses no juice. The contents of different cells are at different pressures. Those which have the lowest hydrostatic pressure when fully turgid lose water first when pressure is applied to the tissue. The pressure at which exudation commences (or more strictly the pressure at which the extrapolated volume-pressure cuts the base line) equals the hydrostatic pressure at full turgor of those cells having the lowest pressure (see also the Discussion, p. 353).

Table 1. *Quantities and osmotic pressures of juice obtained by small pressure increments on leaves of* Fagus sylvatica purpurea

Exp. no.	Applied pressure (atm.)	Osmotic pressure of juice (atm.)	Volume of juice (% fresh weight)	Colour
VIII, live	43	2·53	16·3	o
	54	3·68	22·5	o
	67	3·86	27·5	o
	103	4·25	33·1	o
	174	3·75	46·0	o
	245	7·15	47·5	+
	353	12·8	54·0	+++
	571	15·3	60·0	+++
	1007	23·1	69·0	++++
VIII, killed residue	1007	27·2	6·3	++++
XI, live	22	2·95	3·3	o
	33	3·95	10·5	o
	46	4·20	15·0	o
	81	4·40	22·4	o
	124	5·3	28·0	o
	183	9·4	32·6	o
	407	21·1	41·0	+++
XIII, live	28	4·5	5·4	o
	38	4·2	10·8	o
	54	3·8	16·1	o
	68	2·4	19·3	o
	106	2·5	24·0	o
	146	2·4	27·5	o
	162	5·9	30·0	+
	248	9·3	34·0	+++
XIII, killed residue	242	28·4	5·1	++++

Columns 3 and 5 refer to the increment of juice, column 4 to the total quantity pressed out at the pressures given in column 2.

No plasmolysis was observed when tissue was immersed in sucrose of 21 atm., and about 10 % of the epidermis was plasmolysed in 23 atm. and 50 % plasmolysed in 25 atm. The mean plasmolytic value of the palisade and spongy mesophyll was 33–39 atm. The coincidence between the value of 21 atm. for the hydrostatic

pressure and 21–23 atm. for the plasmolytic value of those cells having the lowest values is striking.

Fagus sylvatica, Exps. VIII and XI. Certain results of these experiments are given in Table 1. They resemble Exp. XIII very closely in most features. The values of the minimum hydrostatic pressure obtained by extrapolating the volume pressure back to zero volume were 26 and 20 atm. respectively, but examination of the data in Table 1 will show that the former figure is rather unreliable owing to the length of the extrapolated part of the curve.

In each of these experiments there is a break in the volume-pressure curve. The extra quantities of juice which are responsible for the "break" contained anthocyan and had higher osmotic pressures than the colourless juice obtained before the break. The associated breakdown of the protoplast takes place at different pressures in different experiments. In Exp. XIII the break occurred at 150 atm., in Exp. VIII at 220 and in Exp. XI at 380 atm. Consequently it appears to be a more or less fortuitous effect of the gradually increasing shearing of the leaves which occurs even in the most carefully prepared piles when the pressure rises as high as 1000 atm. It should be emphasized that the breakdown may occur at very low pressures when slipping and shearing of the leaves occurs as a result of careless packing or movement of the plates between which they are pressed.

Gossypium barbadense, Exps. XXII and XXIV. The most remarkable contrast between our results and those of Phillis & Mason (1937) is that, whereas they obtained no exudation of juice from living cotton leaves when pressures as high as 280 atm. were applied, we find marked exudation from beech leaves at applied pressures of 20–30 atm. There is no doubt that we have succeeded in applying these and much higher pressures also without causing any marked injury to the tissue, for the normal semi-permeability of the protoplast has remained unimpaired. We felt it desirable to apply our technique to cotton leaves.

The leaves of American Upland Cotton used by us were grown in the Manchester University Experimental Grounds, and we wish to thank Prof. Drummond for the supply of these. The extreme temperatures in the greenhouse where they were grown were probably about 18–40° C.

Triangular pieces were cut out between the main veins, and piles of these triangles, cut down so that all were the same area and exactly superimposed, were placed between the steel plates in the testing machine as usual.

Certain detailed results for these two experiments are given in Table 2, and the results of Exp. XXIV are graphically expressed in Fig. 2. It will be noted that juice is expressed from these cotton leaves at even lower pressures than are required to express juice from copper-beech leaves. Extrapolation of the volume-pressure curve back to zero volume indicates first traces of exudation at about 8 atm. corresponding to a hydrostatic pressure of the cell contents of this value for those cells having the lowest hydrostatic pressure.

Plasmolytic data of significance in this connexion were as follows: palisade 3·1 % plasmolysed in 16·6 atm., spongy mesophyll 20·0 % plasmolysed. No plasmolysis was observed in solutions of 12 atm. osmotic pressure. The minimum osmotic

values determined plasmolytically were therefore some 50–100% greater than the hydrostatic pressures within the cells determined mechanically. We are inclined to ascribe this difference to experimental error in the mechanical determination of the hydrostatic pressure. As pointed out earlier, the pressure applied calculated from the weight and total area is lower than that which acts on the most highly compressed parts of the tissue. The pressure applied to cells from which the first traces of juice exude may therefore be a few atmospheres greater than the calculated mean applied pressure. That this is so is confirmed by later experiments in which other

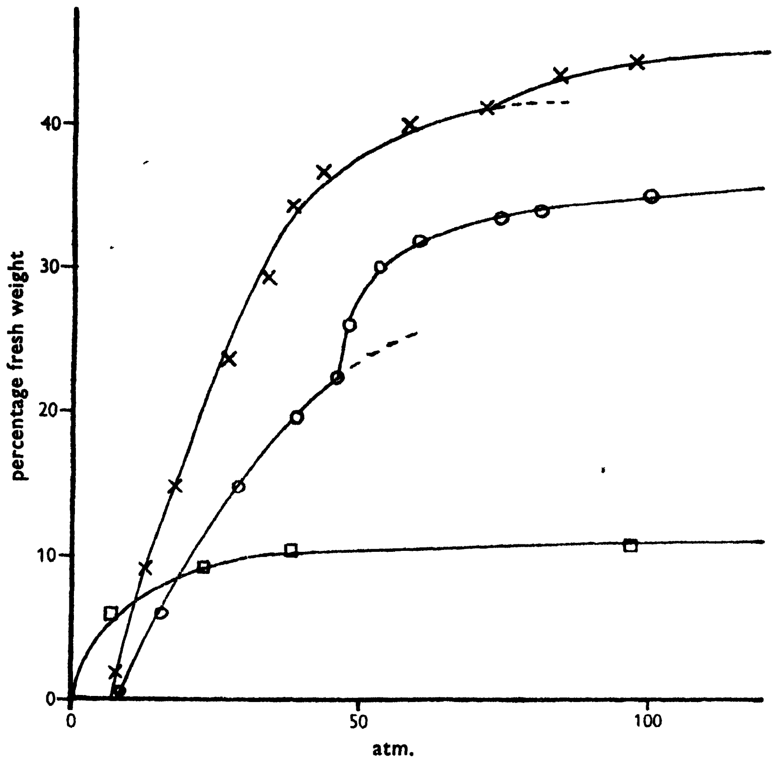

Fig. 2. Volumes of juice expressed by small pressure increments from leaves of *Gossypium barbadense*, Exp. XXIV: living leaves —×—; killed residue —□—; and from leaves of *Parthenocissus quinquifolius*, Exp. XIX: living leaves —○—.

independent determinations of the hydrostatic pressure are compared with those based on the backward extrapolation of the volume-pressure curves.

The data for the two experiments which are presented in Table 2 show essentially the same features as are shown by beech leaves. The osmotic pressures of the expressed juice were relatively high, 4–6 atm. The osmotic pressure of juice obtained after the break in the curve was 9·5 atm. The residue from this pressure treatment was killed and a further quantity of juice of 21–26 atm. was obtained.

A feature which deserves comment is that in the range 8–40 atm. the relation between applied pressure and volume of juice expressed is nearly linear.

Table 2. *Quantities and osmotic pressures of juice obtained by small pressure increments on leaves of* Gossypium barbadense

Exp. no.	Applied pressure (atm.)	Osmotic pressure of juice (atm.)	Volume of juice (% fresh weight)
XXII, live	11·2	—	0·0
	14·5	5·6	6·0
	22		13·3
	30	—	16·4
	45	5·8	21·9
	84		28·0
	108	—	31·1
XXII, killed residue	7·8	20·0	11·1
	18		13·9
	78		17·8
XXIV, live	8	—	2·2
	13	5·9	9·7
	19	5·5	14·7
	26	4·5	23·6
	32	4·4	29·4
	44	5·8	36·5
	97	9·5	44·2
XXIV, killed residue	7	22·1	6·1
	24	26·1	9·2
	97		10·8

Similar data for leaves of Prunus laurocerasus

Exp. no.	Applied pressure (atm.)	Osmotic pressure of juice (atm.)	Volume of juice (% fresh weight)
XXIII, live	10	—	0·0
	16	2·0	2·1
	20		4·2
	26	0·75	7·8
	36	0·18	12·0
	54	0·30	17·7
	108	0·30	21·6
	154	0·65	23·0
XXIII, killed residue	18	29·5	8·2
	37	36·7	11·0
	94	34·6	13·9
	218	37·4	17·3

Prunus laurocerasus, Exp. XXIII. Data relating to this experiment are given in Table 2 and Fig. 3. Various points call for comment. The first sample of juice was collected at an applied pressure of 16 atm. Between this and 50 atm. the relation between applied pressure and volume of juice expressed is almost linear. All samples of juice pressed from the unkilled tissue had osmotic pressures well below 1 atm. except the first two which had the value 2·0 atm. These first two samples smelt slightly of HCN, but the other samples from the live tissue scarcely smelt and gave no sodium picrate reaction.

After killing, a further fairly large amount of juice was expressible: this juice was orange and smelt very strongly of HCN and gave a very strong picrate reaction. Its osmotic pressure was high (29–37 atm.).

It seems very clear that using this material it is possible to press out of the live tissue water of fairly high purity. In this experiment the total quantity which could

be expressed was about 23 % of the fresh weight; the shape of the curve suggests that higher pressures than those applied would not have caused any appreciable extra exudation. The ready explanation of this is that almost all the vacuolar water has been pressed out, and this is borne out by the appearance of the cells.

In many other experiments it has been found that it is not possible to apply pressures above about 150–170 atm. to living cherry-laurel leaves, since the leaves tear and the piles collapse and become sheared resulting in death of the tissues. Pressures over 3000 atm. can be applied to beech leaves without this mechanical breakdown occurring.

Sections were cut from leaves which had been subjected to pressures of 150 atm. and were mounted in either paraffin or water. The latter recovered completely and the individual cells were indistinguishable from those from unpressed tissue. The

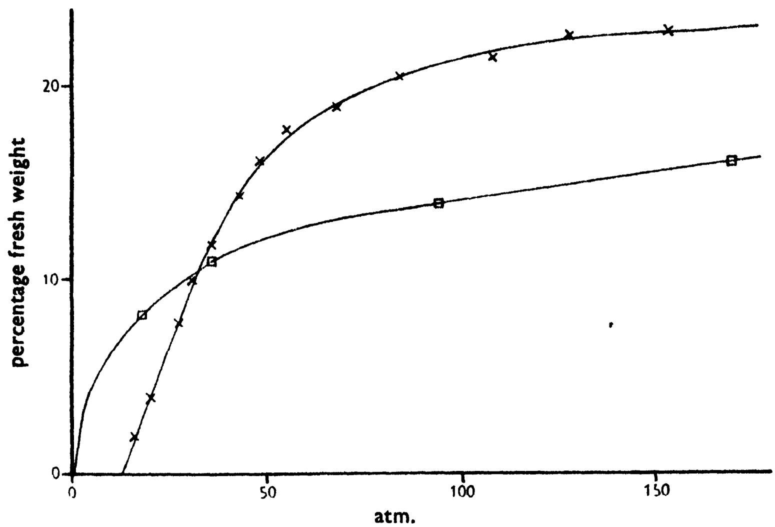

Fig. 3. Volumes of juice expressed by small pressure increments from leaves of *Prunus laurocerasus*, Exp. XXIII. Conventions as in Fig. 1.

cells of "pressed and recovered" tissue could be plasmolysed, and it was found that the plasmolytic value was approximately the same as that of fresh unpressed tissue. Sections mounted in paraffin had also partially recovered because directly the pressure was released the compressed cells absorbed water which had been pressed into the intercellular spaces and spaces between and around the leaves. Their cells therefore had small vacuoles which may have reformed when the pressure was released, or possibly the view expressed earlier, that the vacuoles are completely expressed, is incorrect.

Extrapolation of the volume-pressure curve gives 12 atm. as the minimum hydrostatic pressure. The corresponding plasmolytic data are as follows: epidermal tissues have lower plasmolytic values than mesophyll, 23 % were plasmolysed at 19·4 atm. and none plasmolysed at 16·6 atm. The mechanically determined hydro-

static pressure is again several atmospheres lower than the plasmolytic value. It seemed important to find out whether this was in fact due to an unfortunately large error in the estimation of the applied pressures.

The major possibilities of error involved in using the testing machine in the manner described are: (*a*) that incorrect centring may result in higher than the estimated pressures being applied at certain regions; (*b*) that waviness of the leaves and the presence of veins may cause the area of contact to be considerably less than the total area of the piece of leaf used, making the calculated pressure too low: this will probably obtain only when very low pressures are applied as in the plotting of the early part of the volume-pressure curve; (*c*) that the area which carries the weight, namely, the cross-sectional area of the mesophyll cells, is less than the total area of the piece of leaf.

The first two errors are largely eliminated by the very simple device for determining hydrostatic pressures illustrated in Fig. 4. The ½ in. steel rod *b* has a wooden platform fixed on the upper end on which weights can be placed. It is mounted accurately vertical and slides freely in the bearings *a*. The ends of the moving rod *b* and the fixed one *c* are cut off at right angles, and, owing to difficulty in accurate machining, two short brass cylinders ½ × ½ in. are placed between the ends of the steel rods. The surfaces of these brass cylinders in contact are ground in with fine emery and rouge. A round piece of leaf cut out by a sharp cork borer is put between the brass cylinders and is examined by the horizontal microscope. Weights are then loaded on to the table at the top of the rod *b* and the tissue becomes compressed.

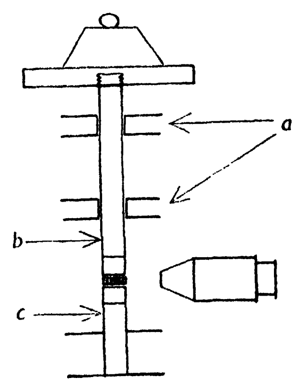

Fig. 4. Apparatus for determination of hydrostatic pressure of cell contents. Description in text.

The use of a rather long rod *b* eliminates the error due to faulty centring. The use of a single piece of leaf as small as 0·19 sq. in. area largely eliminates errors due to lack of flatness of the tissue. Assuming that the whole area of the piece between the brass plates is subjected to pressure, a load of 1 kg. corresponds to 0·81 atm. (with our apparatus) and loads up to 80 kg. (= 1·5 cwt. = 65 atm.) can be applied.

Microscopic examination of the cut surface with suitable surface illumination shows that as the pressure is increased the air spaces of the spongy mesophyll become decreased in volume, and at 16 atm. the thickness of the leaf had decreased to about half. No trace of exudation of water or juice could be observed at this applied pressure. The first traces of exudation are very readily seen with suitable illumination and are regularly obtained with applied pressures between 18 and 19 atm. in all cases. The deformation of the cells at this pressure is not sufficient to press out the intercellular spaces completely, and such spaces as remain become injected with water pressed out of the cells. Further increase in pressure causes expression of more water from the cells and also from these intercellular spaces to

the outside. On removal of pressure the expressed water is reabsorbed into the cells from the intercellular spaces.

The pressure applied has been calculated on the assumption that the area which carries the weight is equal to the total area of the piece of leaf. Actually it would be more accurate to treat the mesophyll as a number of cylinders closely packed. The ratio of the area of cross-section of cylinders to the total area when they are close-packed is $\pi : 2\sqrt{3}$ or 91 : 100, so it would seem that the actual area carrying the weight is about 10% less than the total area of the piece of leaf, and the calculated pressure is therefore about 10% too low. The estimated hydrostatic pressure without making this allowance is 18·5 atm., and making this allowance for the intercellular air spaces it is 20·5 atm.; 20% of the epidermal and none of the mesophyll cells showed limiting plasmolysis in a solution of 19·4 atm. This very close agreement justifies the view that here the plasmolytic value is equal to the hydrostatic pressure of the cell contents.

Parthenocissus quinquifolius, Exp. XIX. Red autumn coloured leaves of the Virginian creeper were used. Vacuoles of mesophyll cells were densely pigmented and those of epidermal cells were pale in colour. The effect of pressure was again similar; first traces of exudation occurred at 8 atm., the exuding juice had an osmotic pressure of 0·7 atm. and was very nearly colourless. A break in the curve (cf. Fig. 2) is seen at a pressure of 40 atm. and the samples of juice exuding after this pressure were coloured dark red by anthocyan. The osmotic pressures of the coloured samples of juice obtained after the break at 40 atm. applied pressure were in between 4·3 and 7·3 atm. higher.

No plasmolysis was observed in plasmolytica of 7·0 atm., 50% of the epidermis and none of the mesophyll showed limiting plasmolysis in 9·3 atm. There is close correspondence between the roughly estimated hydrostatic pressure and osmotic "value" obtained by plasmolysis.

Beta vulgaris, var. Crimson Globe, Exp. XXIX. The vacuoles of the mesophyll cells were strongly coloured and those of the epidermis were for the most part uncoloured or very pale. Extrapolation of the volume-pressure curve gave first traces of exudation at 10 atm. Microscopic observation of a single piece of leaf in the apparatus described on p. 347 indicated first traces of exudation at 11·0 atm. The two methods are in good agreement, and the hydrostatic pressure indicated should be compared with the plasmolytic data. 3% of the epidermal cells showed limiting plasmolysis in 9·5 atm. and 90% in 10·8 atm. No mesophyll cells were plasmolysed in either of these solutions.

The juice pressed from the leaf was a very faint pink, but its osmotic pressure was less than 0·05 atm. It is therefore nearly pure water. Breakdown of the tissue occurred at the rather low applied pressure of about 40 atm.

EFFECTS OF QUICK INCREASE OF PRESSURE ON TISSUES

Experimental methods. Piles of leaves were prepared as in the slow-pressure treatment and were placed between tin-foil covered steel plates in the testing machine. After fitting up, the initial pressure on the pile of leaves was about 2 or

3 atm. The pressure was raised from this low value to a pressure of 100–150 atm. within 1 min. Juice exuded very rapidly at first, and when exudation stopped the pressure was again raised by another large increment of say 100 atm. applied suddenly. A few typical experiments will be dealt with in detail.

Fagus sylvatica purpurea, Exp. XII. Copper-beech leaves from the same tree as that used in the slow-pressure experiments were used. They were subjected to the pressures indicated in Table 3. The pressure was raised from about 2 to 156 atm. suddenly and, after all the exudation had ceased, another sudden increase to 330 atm. was applied, and later another sudden increase to 412 atm. Since the volume-pressure curve was now approaching an asymptote no further increase was applied, and after all exudation of juice had ceased the pile of leaves was taken out of the press, a leaf was removed for examination, and the remainder were killed by freezing with solid carbon dioxide, and after thawing they were pressed again with applied pressures of 156, 330 and 412 atm. A further fairly large quantity of juice was pressed out from the killed residue.

Data regarding quantities, osmotic pressures, and colours of these samples are given in Table 3. The most noteworthy point is that the samples obtained from the live tissue are rather strongly coloured by anthocyan and have the relatively low osmotic pressure of 8–12 atm. Samples obtained from the residue after killing were poor in anthocyan and had osmotic pressures of 24–25 atm. Approximately 80% of the total anthocyan was contained in the three samples of juice pressed from the living tissue. The effect of this on the appearance of the leaves was very striking, as they were uninjured and quite green.

Sections were examined mounted in paraffin and also mounted in water. The former were about half the thickness of the unpressed leaf, but the latter had swelled up and recovered to a thickness very close to that of the unpressed leaves. For the most part the cells did not appear to be injured, but they no longer contained coloured vacuoles and indeed colourless vacuoles could not be detected. This apparent absence of vacuoles is reported by Mason & Phillis for cotton leaves which had been quickly pressed and were then allowed to recover in water. Their view that the cytoplasm swells and fills the cell is supported by our observations on copper-beech leaves.

It is impossible to escape from the conclusion that this quick pressure treatment has pressed out the vacuolar sap almost completely. There is no doubt that both water and the very large anthocyan molecules have been pressed out. There is, of course, no direct evidence of the presence of any other substances in the vacuoles or of their being pressed out, but it seems to us highly improbable that anthocyan should be pressed out and that other vacuolar solutes should be filtered off by the cytoplasm. The osmotic pressure of this expressed vacuolar sap varied from 8 to 12 atm.

Mason & Phillis report the pressing out under similar conditions of juice from cotton leaves which they regard as vacuolar sap and which also had the very low osmotic pressure of 2–3 atm. They regard it as being pressed out through "fissures in the cytoplasm", a view with which we agree if our notions of the nature of a cytoplasmic fissure are the same.

That this expressed juice is pure vacuolar sap is not claimed, though we believe that the extent to which it is contaminated is not great. To some extent it is likely to be contaminated by water pressed out of cells in a similar manner to that observed when the applied pressure is raised slowly. A few cells are almost certain to be sheared and killed, and the sap pressed out of them also contaminates the vacuolar sap.

After all the juice expressible from the live tissue had been pressed out, the tissue was killed and pressed again. More juice exuded and the part of the tissue from which the water originated was the cytoplasm. This juice which we are calling cytoplasmic sap contains a much smaller concentration of anthocyan than the alleged vacuolar sap, but its osmotic pressure is more than twice as great (24–25 atm.). It seems probable to us that the anthocyan in this fraction is due to contamination by vacuolar sap which is incompletely pressed out of the intercellular spaces or which remains incompletely pressed out of the vacuoles.

Plasmolytic values of the epidermal, palisade, and spongy mesophyll cells are about 25, 36 and 33 atm. respectively. The method used to obtain from these data a figure for the "mean osmotic value" of the whole leaf is discussed later. On the classical theory to which we alluded in the introduction one might expect from these plasmolytic values that the mixed expressed sap from the whole leaf would have had an osmotic pressure of 30–32 atm. Actually the mixed entire cell sap has an osmotic pressure of 15 atm. and that of the alleged vacuolar sap is about 8 atm.

Table 3. *Quantities and osmotic pressures of juice obtained by large increments of pressure on leaves of* Fagus sylvatica purpurea

Exp. no.	Applied pressure (atm.)	Osmotic pressure of juice (atm.)	Volume of juice (% fresh weight)	Colour (Lovibond units per mm. thickness)		
				Red	Blue	Yellow
XII, live	156	7·87	9·1	2·2	2·2	3·0
	330	10·65	16·5	—	—	—
	412	11·62	18·2	—	—	—
XII, killed residue	156	24·00	6·5	1·0	0·2	0·6
	330	24·7	9·7	—	—	—
	412	24·95	10·1	—	—	—
VII, live	180	7·36	20·2	—	—	—
	400	8·50	37·1	2·15	1·80	0·48
VII, killed residue	39	15·44	5·1	0·99	0·18	0·27
	156	18·45	16·8	0·42	0·18	0·48
	390	19·15	25·5	—	—	—
	2300	19·70	35·3	—	—	—
VI, live	173	6·6	10·4		+++	
	412	8·0	19·1		+++	
VI, killed residue	165	18·7	12·5		+	
	412	19·6	17·5		+	
	3700	19·4	26·0		+	
XIV, live	101	10·95	16·0	6·2	3·0	3·0
	133	12·90	17·2	—	—	—
	166		19·4	—	—	—
XIV, killed residue	67	23·15	5·6	3·5	0·5	1·6
	133	24·35	8·0	—	—	—
	166	—	8·1	—	—	—
	1000	—	8·2	—	—	—

Fagus sylvatica purpurea, Exps. VI, VII and XIV. These experiments were carried out in the same manner as Exp. XII which has been described, and the results which are given in Table 3 are similar to those of Exp. XII and consequently require no further comment at this point. It is made clear by the results of Exp. VII that the first sample of juice pressed from the killed residue left after pressing out of the "vacuolar" sap is more darkly coloured with anthocyan than subsequent samples. This first sample also has a lower osmotic pressure than the later samples, and it therefore seems probable that it is contaminated by the vacuolar sap which has not been completely pressed out of the intercellular spaces and that it partially washes away the contaminant.

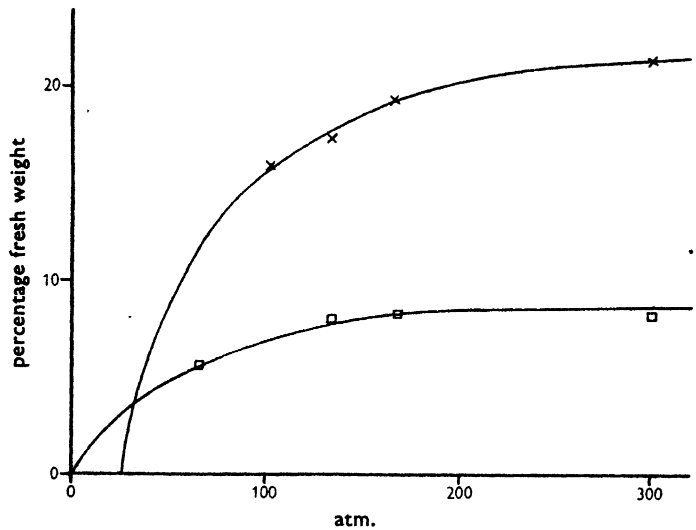

Fig. 5. Volumes of juice expressed by large pressure increments from leaves of *Fagus sylvatica*, Exp. XIV. Conventions as in Fig. 1.

The volume-pressure relations obtained in Exp. XIV are shown graphically in Fig. 5 which may be compared with the very similar volume-pressure relations obtained when the small increments of pressure are used.

Parthenocissus quinquifolius, Exp. XVII. Autumn coloured leaves similar to those used in the slow-pressure treatment were employed. Results are given in Table 4. It will be seen that vacuolar pigment is pressed out by the sudden rise of applied pressure from 12 to 81 atm. A further amount is pressed out by the next increment of 60 atm. These expressed saps have low osmotic pressures of 5–7 atm.

The residue from this treatment after killing yields a further supply of sap of osmotic pressure = 25·2 atm. This supposedly cytoplasmic sap also contains anthocyan pigment which colours it almost as darkly as it colours the vacuolar sap pressed out of the live tissue. One might have expected a less pigmented sap from the killed residue as is found when copper-beech leaves are used. The approximately equal pigmentation should probably be ascribed to the fact that the volume of the cytoplasm is relatively small compared to that of the pigmented vacuoles,

whereas in the copper beech the volume of the cytoplasm is large. Contamination by vacuolar sap therefore causes much greater change in the composition of the cytoplasmic sap of the Virginian creeper than of the copper beech.

Beta vulgaris, Exp. XXX. It was found to be impossible to apply an increment of pressure of more than 40 atm. to these leaves without complete breakage of the tissues. Application of 40 atm. expressed anthocyan-containing sap having an osmotic pressure of 7·00 atm. Another sample of similar leaves was first killed by freezing in solid carbon dioxide, and on pressing it yielded a similar sap having an osmotic pressure of 8·8 atm. The mean plasmolytic value was 14·5 atm. for the mesophyll and 10·2 for the epidermis. The pigmentation of the two samples of sap was nearly equal.

Gossypium barbadense, Exp. XXV. Similar leaves to those used in the slow-pressure treatment were used. Results are given in Table 4. Owing to absence of vacuolar pigment or of any certainly established vacuolar constituent, we have no direct evidence in this case of the origin of the saps pressed from the live and dead tissues.

Prunus laurocerasus, Exp. XXVIII. Similar material to that used in the slow-pressure treatment was used in this experiment, the results of which are given in Table 4. The most notable feature of the experiment is that quite a considerable increment of pressure expresses a juice having an osmotic pressure of only 0·35 atm. This juice is almost certainly water which is pressed through the cytoplasm, and it is very nearly as pure as the water pressed out in the course of the slow-pressure treatment. In fact this quick-pressure treatment yields results much the same as those yielded in the slow pressing.

Attempts were made in other experiments to press out vacuolar sap by using

Table 4. *Quantities and osmotic pressures of juice obtained by large increments of pressure on leaves of* Parthenocissus, Gossypium, *and* Prunus laurocerasus

Exp. no.	Applied pressure (atm.)	Osmotic pressure of juice (atm.)	Volume of juice (% fresh weight)	Colour (Lovibond red units per mm. thickness)
Parthenocissus	14	—	1·5	—
XVII, live	81	5·7	18·3	16·0
	141	6·9	25·1	21·0
XVII, killed residue	136	25·2	5·8	18·0
Gossypium	12	—	0·01	—
XXV, live	41	9·2	18·5	—
	102	10·6	27·4	—
XXV, killed residue	3	16·6	12·1	—
	17	18·2	18·2	—
	97	—	22·6	—
				HCN
Prunus	16	—	0·9	0
XXVIII, live	78	0·35	16·1	0
	134	1·55	18·7	0
XXVIII, killed residue	12	21·5	12·3	+++
	36	24·8	17·1	+++
	124	25·7	23·2	+++

larger increments than those used here, but in all cases either almost pure water was pressed out or the applied pressure was so great that the cells broke and were killed.

Hedera helix var. *purpurea*, Exp. XXXII. The "purple-leaved" ivy contains anthocyan pigment in the palisade and spongy mesophyll cells but not in the epidermis. This pigment is present in the vacuoles. It was found to be impossible to press out the pigment from living leaves. The sap expressed from living leaves is nearly pure water, and when the residue from this treatment is killed a further quantity of sap can be pressed out which is rich in anthocyan.

The failure to press vacuolar sap from living ivy or cherry laurel would presumably be remedied if a pressure increment larger than 150 atm. could be applied, but we have never succeeded in applying a sufficiently large pressure increment without shearing and destruction of the tissues.

Discussion

(*a*) The cells of leaves while attached to the parent plant are not in equilibrium with an external water phase at normal pressure but with water which is maintained in a state of tension (i.e. at a reduced pressure) by the process of transpiration. In consequence the leaf cells are not fully turgid and they tend to absorb water with a pressure, termed the suction pressure, which is equal to and acts in the opposite direction to the tension in the column of water external to the protoplasts.

When the leaves are cut the tension in the water columns outside the protoplasts is probably reduced, and a certain amount of water thus becomes absorbed into the protoplasts. Consequently the hydrostatic pressure of the cell contents is slightly raised and the suction pressure correspondingly slightly reduced. It should be emphasized that the water external to the protoplasts in such cut tissue is still in tension (in general) because the capillary and ultra-capillary spaces of the cell walls are not completely saturated.

It would probably be more strictly accurate to write of the suction pressure of the protoplast (i.e. cytoplasm + vacuole) which naturally is reduced the more the protoplast is compressed by the stretched cell wall. This suction pressure is opposed by what is most conveniently termed the osmotic water attraction of the cell wall. This osmotic water attraction (the term is applied in a similar sense to solutions and pure substances by Schreinemakers) is caused largely by the lack of saturation of the wall with water.

The effect of pressing normal leaves, which are in the condition just described, is to cause a deformation of the cells of the tissue which become flatter with curved side walls of smaller radius of curvature than formerly. The wall pressure is therefore increased and the hydrostatic pressure of the cell contents is increased. A flow of water out of the cell results which causes the cell wall to become more nearly saturated and the tension of the water in the wall decreases. As the applied pressure increases the walls become completely saturated and finally water exudes out of the walls into the intercellular spaces. The total quantity of water which must be pressed out of the protoplast in order completely to saturate the cell wall is very small indeed and, if the turgor stretching is negligible, the wall pressure acting when

the wall is thus saturated with water is equal approximately to the wall pressure attained when the cell is made fully turgid by immersing it in pure water. In both these cases the water outside the protoplast with which it is in equilibrium is at normal pressure. Where the degree of stretching is considerable one must take into account the fact that the wall pressure of the cell made turgid by immersion in water is smaller than that of a cell made turgid by application of pressure mechanically.

The tissues of the leaves used by us had a degree of turgor stretching of under 3 %; and such low turgor stretching is in fact usual in leaf tissues. One is therefore involved in negligible error in regarding the wall pressure or hydrostatic pressure of a fully turgid cell immersed in water as equal to that of a non-turgid cell pressed to the stage at which the wall has become fully saturated with water. This assumption is made throughout this discussion.

When the stage has been reached at which the wall is fully saturated, the wall pressure equals the pressure with which the protoplast tends to absorb water. This *water absorption pressure*, as we propose to term it, has been regarded on the classical theory as identical with the osmotic pressure of the cell contents. Under the circumstances described the pressure exerted by the plates of the pressing machine is also equal to the hydrostatic pressure of the contents which equals the wall pressure. In our terminology the suction pressure of the cell equals the water absorption pressure minus the wall pressure.

An addition to the pressure exerted by the plates of the testing machine deforms the tissue still more, thus increasing the wall and hydrostatic pressures which become greater than the water-absorption pressure. Water is consequently pressed out and the increased concentration of the cell contents results in greater water-absorption pressure which eventually balances the wall pressure.

If the water absorption of a cell were really due to the osmotic pressure of the vacuolar sap and the water-absorption pressure were identical with the osmotic pressure of this sap, there would be an approximately linear relation between pressure and concentration of the sap. Solutions of electrolytes exhibit this linear relation fairly closely, but non-electrolytes, especially those which have large molecules such as sucrose, show quite considerable divergences, and of course the divergences are always great with very concentrated solutions.

An approximately linear relation between pressure set up and the concentration is equally probable if the pressure within the cell is due to a diffusion effect of the type referred to as "anomalous osmosis".

The experimentally observed relations between applied pressure and the volume of juice exuded are in close agreement with the expectations based on this discussion. This may be made clearer by consideration of the calculated curves given in Fig. 6. The first or upper of these curves gives as ordinates the percentage of vacuolar water removed by the pressures shown as abscissae where the water-absorption pressure (osmotic pressure of the classical theory) is taken as 20 atm. and all the cells are assumed to have this same value. A negligible volume of water is pressed out at pressures lower than that required to saturate the wall; i.e. at pressures lower

than the hydrostatic pressure of the fully turgid cell which equals approximately the water absorption pressure of the vacuole. Above this pressure the relation between pressure and volume is hyperbolic as shown.

The assumption made here that all the cells of the tissue have the same water-absorption pressure is obviously not even approximately true in most leaves. A considerable range of values occurs and, probably because the modal values for the four major tissues of the leaf are different, the frequency distribution of these values is very irregular and very divergent from a normal binomial distribution. Actually we can assume without very great error equal frequency of each value within the range found. The lower curve in Fig. 6 gives the percentage of vacuolar water pressed from leaves, the cells of which have water-absorption pressures between 20 and 40 atm. distributed with equal frequency as described. The curve is similar in

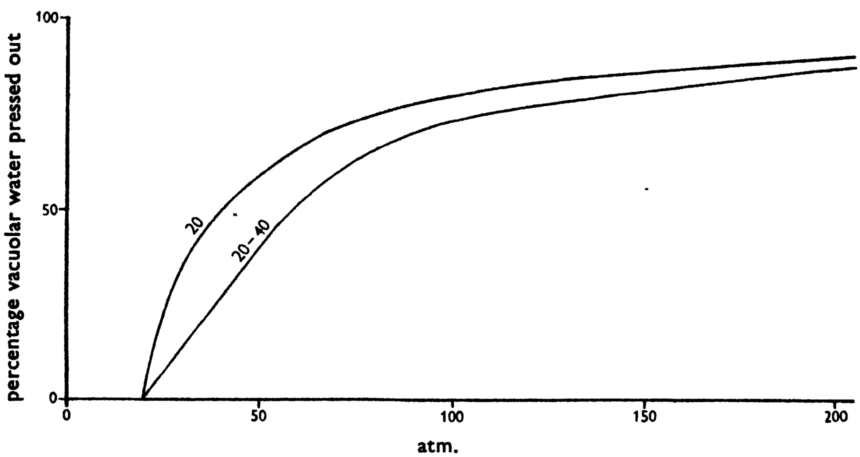

Fig. 6. Theoretical curves showing percentage of vacuolar water expressed from tissue at pressures shown as abscissae. Description in text.

shape to those obtained experimentally. The almost linear phase where the expressed volumes are small is an expression of the almost linear frequency distribution of the water-absorption or hydrostatic pressures of the cells of the leaves.

We have thus a direct mechanical method of estimating the hydrostatic pressure at full turgor of the cells of the leaf having the lowest pressures. So far as we are aware estimations of the hydrostatic pressure of plant tissues have not been made before except by Dixon (1896*a*), who compressed complete leafy branches enclosed, apart from the protruding piece of stem, in strong glass cylinders which could be filled with compressed gases, usually carbon dioxide or air. The pressure at which the leaves collapsed was interpreted, at that time, as being equal to the osmotic pressure of the leaf cells, but the actual osmotic pressure of the expressed juice was not in this case determined. The use of plasmolytic methods involves the acceptance of the hitherto unproved assumption that the hydrostatic pressure equals the external osmotic pressure at limiting plasmolysis.

(*b*) It has, in general, been assumed that the hydrostatic pressure of the contents of a fully turgid cell is simply connected with and may be identical with the plasmolytic value. Thus P_t, the hydrostatic pressure of the fully turgid cell (=its osmotic pressure on the classical theory), is related to P_l, the osmotic pressure of the plasmolyticum at limiting plasmolysis by the equation

$$P_t = P_l \frac{V_l}{V_t},$$

where V_l and V_t are the volumes of vacuole at limiting plasmolysis and full turgor respectively. V_l/V_t, the degree of turgor stretching, may be very close to unity. The plasmolyticum must be inert and unable to permeate the protoplast, and in practice it may not be possible to decide with certainty that a given plasmolyticum fulfils these conditions.

We are describing as the *minimum plasmolytic value* of a tissue the osmotic pressure which causes limiting plasmolysis in not more than 5% of the cells. This value, it will be seen from Table 5 (p. 357), is very close to the minimum hydrostatic pressure determined directly. The term *minimum hydrostatic pressure* refers to the fully turgid tissue. It is the pressure of the contents of those cells whose hydrostatic pressure at full turgor is the smallest. We have no means of determining the hydrostatic pressure of a cell which is not fully turgid.

In plasmolysis the water-absorbing power of the protoplast is balanced by the osmotic water attraction of the plasmolyticum.

(*c*) This, being true of those cells having the lowest plasmolytic values, is presumably true of all; hence the mean plasmolytic value should equal the mean hydrostatic pressure at full turgor. This can be compared with the osmotic pressure of the mean expressed sap or mean vacuolar sap. The *plasmolytic values* of epidermal and mesophyll tissues are taken as the osmotic pressures of plasmolyticum in which 50% of the cells show plasmolysis or limiting plasmolysis. The 50% value is obtained from the S-shaped curve relating percentage plasmolysis with osmotic pressure of plasmolyticum as formerly described (Bennet-Clark *et al.*). The *mean plasmolytic value* of the whole leaf is obtained from similar data, but it is obvious that there are special difficulties in assessing it because limiting plasmolysis can be most certainly observed in epidermal tissues in surface sections and in palisade in vertical sections. Moreover, if the basis were the relative numbers of cells of each tissue, the fact that the large epidermal cells have usually a lower plasmolytic value than the smaller mesophyll cells would cause the 50% value to be greater than the expected osmotic value of the "mean juice" on the basis of the classical theory. In order to get a comparable plasmolytic value one has to take into account the volumes of the several tissues. The mean plasmolytic value used by us is therefore given by the expression

$$P_{(\text{mean})} = \frac{P_{(\text{ep.})} V_{(\text{ep.})} + P_{(\text{pal.})} V_{(\text{pal.})} + P_{(\text{sp.})} V_{(\text{sp.})}}{V_{(\text{total})}},$$

where the P and V terms are the plasmolytic values and relative volumes of the several tissues. Mean plasmolytic values in this sense are cited in Table 5 (p. 357);

they are slightly lower than the mean plasmolytic value as recorded in the usual manner where only the numbers and not the volumes of the cells are considered.

(*d*) *Entire cell sap* is removed by killing (by freezing with solid carbon dioxide in this study) and pressing. Most workers have followed the lead of Dixon & Atkins (1913) in using this method to obtain sap of reliable origin. Osmotic pressures were obtained micro-cryoscopically.

(*e*) So far as most cell constituents are concerned we have no certain information as to their distribution between vacuole and cytoplasm. Certain cells can be seen to have colourless cytoplasm and fair concentration of anthocyan pigments in the vacuole. The greater part of the anthocyan is expressed by the "quick-pressing" of living leaves as are water and certain other solutes. It seems improbable that any vacuolar solutes likely to affect the osmotic pressure are filtered off by the living cytoplasm, since the anthocyan molecule is very large (molecular weight *ca.* 500–800) and very insoluble in lipoid and is therefore more likely to be retained than most cell constituents.

We therefore agree with Mason & Phillis in regarding the quick-press juice as *vacuolar sap*, although as pointed out on p. 349 it seems probable that it is contaminated to some extent.

Similarly we follow Mason & Phillis in describing as *cytoplasmic sap* the juice pressed from the residue left after expression of the vacuolar sap. This cytoplasmic sap can be pressed out of the residue only when it has been killed. Possibly application of a sufficiently large pressure to living cells might result in the expression of water and other constituents, but we have never succeeded in raising the pressure above 3000 atm. without breaking the cell walls, and this is of course associated with death of the cell and expression of the entire cell contents. At pressures lower than this a large amount of water and solutes remains in the living cell (from which the vacuole has almost disappeared) and this becomes readily expressible at very low pressures when the cell is artificially killed. The cytoplasmic sap is undoubtedly contaminated to some extent by vacuolar sap.

Table 5. *Summary of experimental results*

	Fagus	*Beta*	*Prunus*	*Parthenocissus*	*Gossypium*, our data	*Gossypium*, Mason & Phillis
Minimum hydrostatic pressure	20–26	9–10	19	9–10	(8–10)	—
Minimum plasmolytic value	19–21	9	18–19	8–9	14–16	—
Mean plasmolytic value	30–32	14	24–25	15–16	22	22
Plasmolytic value:						
Epidermis	21–23	10	20	9–10	20	—
Palisade	33–39	14·5	26	18–20	23	—
Spongy mesophyll	29–32	14·5	26	18–20	22	—
O.P. cytoplasmic sap	18–24	—	—	25	17–18	20
O.P. vacuolar sap	5–8	7	—	5–7	9–10	2–3
O.P. entire cell sap	15	9	12	8–10	12–14	—

In all cases the mean plasmolytic value (=mean hydrostatic pressure at full turgor) exceeds the osmotic pressure of the entire cell sap and very greatly exceeds the osmotic pressure of the vacuolar sap which may be from 50 to 90 % lower than the mean hydrostatic pressure.

(*f*) The water-absorbing power of the cell evidently exceeds the osmotic pressure of the vacuole, and we formerly ascribed this excess absorbing power to some facet of metabolism which we termed secretion of water into the vacuole. It seems desirable to amplify this statement in view of the peculiarities of the system.

The term secretion seems to imply an ability of the living protoplast to override the physico-chemical laws of diffusion for which we have at present no evidence. We feel that the cell should be treated as a diffusion system. It will assist our discussion to consider two simple special cases in which a constant rate of diffusion is maintained during the period of experiment: the system

phthalic acid solution + solid phthalic acid | cellophane membrane | invariant water

maintains diffusion of phthalic acid to the right at a constant rate whilst solid acid remains. Coincidently water diffuses to the left or sets up a pressure in the left compartment; and with a suitable membrane the pressure is much greater than the osmotic pressure of the solution and is quite unconnected with it.

If the cellophane membrane is replaced by one of gelatin the acid diffuses similarly to the right, but in this case water also diffuses to the right or sets up a constant pressure in the right compartment if this can be kept invariant. The nature of the membrane thus determines the magnitude and sign of water movement or pressure.

Electrostatic forces may be the cause of these water movements (cf. Bartell & Hocker, 1916; Freundlich, 1916, 1930–2, or the review by Stiles, 1924), but these movements may equally be effected by ions transporting shells or atmospheres of water molecules. Thus in the first case mentioned above OH^- travelling to the left may carry more water than H^+ travelling to the right. Alternatively, the positive potential of the right side of the membrane relative to the left (maintained by the constant diffusion of acid) may bring about electro-osmosis. This latter and older view of the mechanism of so-called anomalous osmosis has been expunged from certain recent treatments of the subject (Freundlich, 1930–2). Details of the mechanism need not detain us. What is important is that water movement or pressure depends on the congruent diffusion of another component of the system which acts as a "carrier".

Secretion of water into an external medium of more or less unlimited volume is relatively easy to understand, but secretion into a limited enclosed space such as a vacuole demands a special type of mechanism. If a high hydrostatic pressure is to be maintained continuously in the vacuole, there must be continuous diffusion of another component acting as a carrier in the sense mentioned above. Such continuous diffusion across the boundary of the vacuole is possible only if some substance is either produced or immobilized in the vacuole or possibly on the inner surface of the cytoplasm.

Aerobic respiration has been found to be connected with accumulation of solutes (Steward, 1935), and also with the water secretion of transpiring cells (Dixon & Barlee, 1939), and one is thus drawn to see in the cyclical processes believed to occur in oxidative anabolism a mechanism which might bring about continuous diffusion across the vacuolar boundary. With suitably chosen reactants the mechanism given below would cause development of a continued hydrostatic pressure in the vacuole:

A (concentration relatively small) $\downarrow$	$\longleftarrow$ diffusion across boundary	A (concentration relatively large) $\uparrow$
B (concentration relatively large)	$\longrightarrow$ diffusion across boundary	B (concentration relatively small)
Cytoplasmic phase		Vacuolar phase

The occurrence of the chemical change $A \rightarrow B$ in one phase and of $B \rightarrow A$ in the other maintains constant diffusion. The casting of carbohydrate and organic acid in the roles of A and B would form an ideal system for transport of water or maintenance of a pressure. Direct evidence in favour of such a system is hardly obtainable. Interference with aerobic respiratory processes frequently leads to loss of hydrostatic pressure or to vacuolar contraction, but this is as easy to interpret as an effect of decreased vitality or tone as to a specific interference with the mechanism of continuous diffusion.

This mechanism has only been mentioned because the criticism has been made to us in conversation that the maintenance of a hydrostatic pressure in the vacuole in excess of the osmotic pressure is impossible. Moreover, it seemed important to point out that continued transport of water to the vacuole maintaining a pressure there demands some chemical change in the vacuolar phase.

(*g*) In conclusion, one may refer to other aspects of leaf water relations. Secretion of water through leaf cells in transpiration is known to occur (Dixon, 1896*b*), and it has recently been shown that this process is connected with aerobic respiration (Dixon & Barlee). Its occurrence emphasizes still further the inadequacy of the classical theory of cell turgor and cell water relations.

Attempts have been made to "explain" irregular plasmolytic data in terms of "bound" water. We feel that great caution is required in the interpretation of plasmolytic data regarding bound water, permeability, as also of such phenomena as false plasmolysis, vacuolar contraction, and *intrabilität*, which can only receive somewhat unconvincing treatment in terms of the classical theory.

Summary

1. Our results confirm the statements of Mason & Phillis that it is possible to subject leaves to considerable pressures without killing or injuring the cells. Pressure causes juice to exude, and if the increment of pressure bringing this about is large we find, as they did, that vacuolar sap is pressed from the living leaves, and when increase in pressure brings about no further outflow of juice the residue which

is still living contains a large amount of water. After killing it is possible to press from this residue a large amount of the remaining water with very low pressures and the exuding water is accompanied by solutes. It is regarded by Mason & Phillis as cytoplasmic sap.

The origin of these alleged vacuolar and cytoplasmic saps is, we consider, made more certain in some of our work in which leaves having cells with anthocyan-containing vacuoles were used. The alleged expressed vacuolar sap contained in certain experiments up to 80 or 90% of the total anthocyan and the green residue left after its removal, on killing, yielded a cytoplasmic sap very poor in anthocyan.

2. If the increment of pressure used to bring about outflow of juice is small, the juice which is pressed out of anthocyan-containing tissue is colourless and tends towards pure water in composition. Osmotic pressures as low as 0·05 atm. have been observed. Part of the water is pressed out of the vacuoles which can be seen to be very greatly reduced in size and to contain more concentrated pigment. It is impossible at present to estimate how much water is pressed out of the cytoplasm. Vacuolar and other cell solutes are retained in the cell to a large extent under these conditions.

3. The curves relating volume of juice expressed to pressure applied are of characteristic shape (see Figs. 1–3), and when extrapolated to zero volume cut the pressure axis at a pressure which equals the hydrostatic pressure of the contents of those cells whose hydrostatic pressure at full turgor is the lowest. The minimum hydrostatic pressure, as we have termed it, approximately equals the osmotic pressure of the solution required to cause limiting plasmolysis of the most readily plasmolysable cells of the tissue.

4. Numerical data are summarized in Table 5, p. 357. They emphasize very strongly the inadequacy of the classical theory of the water relations of the cell. We point out that a continuous diffusion process is capable of maintaining in one phase of the system a hydrostatic pressure which bears no relation to the osmotic pressure and which may be either greater or less than this. There is at present no evidence as to the nature of any processes of diffusion in the cell which might cause the setting up in the vacuole of a hydrostatic pressure as much as ten times greater than the osmotic pressure. It seems clear, however, that the osmotic pressure of the vacuole coupled with the necessary protoplasmic membrane completely impermeable to solutes is not the sole cause of the observed hydrostatic pressure.

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PROTEIN SYNTHESIS IN MATURE AND SENESCENT LEAVES OF BARLEY

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(With 1 figure in the text)

Introduction

During the life-history of a leaf of an annual plant it has been generally observed that a characteristic sequence of events occurs. In the adolescent stage various attributes including the area, dry weight, and the absolute water and protein contents all increase rapidly and reach maxima. These maxima, however, are not necessarily coincident in time. The dry weight may continue to increase after the leaf is fully expanded, indicating that reserves accumulate (Petrie *et al.* 1939). These are probably in the form of carbohydrates and allied substances, for the percentage sugar content of leaves tends to remain low during rapid growth and to increase after the attainment of maximum area (Gregory & Baptiste, 1936; Barnell, 1936). Further thickening of the cell walls may also account for some increase in dry matter. The stage of development of the leaf at which the maximum protein content is reached varies with the position of the leaf on the plant. Richards & Templeman (1936) found in barley that after full emergence protein was continuously hydrolysed in the lower leaves; while in the last leaves formed (8th, 9th and 10th), which were smaller, proteins accumulated for some time after complete expansion. Unpublished data obtained in this laboratory by Miss Ruth Watson for tobacco leaves indicate that maximum nitrogen content was reached before maximum area.

Various factors have been suggested as determining the protein loss in mature leaves (Petrie, 1937). One factor that must inevitably play a part is the formation of new leaves and, finally, of the inflorescence above the leaf with which we are concerned. These organs, in which protein synthesis is rapid, act as sinks[1] for nitrogen and must increase the rate of translocation of soluble nitrogen from the mature leaves below them.

Other factors may, however, be superimposed upon this. Thus the leaf may completely lose the power of protein synthesis at maturity. In two series of experiments on detached leaves floated on nutrient solutions, Pearsall & Billimoria (1938) found that in dicotyledons protein synthesis occurred only in young immature leaves, and in *Narcissus* leaves only in the basal, elongating portion. Mature leaves appeared to be incapable of synthesizing protein under the conditions of their experiments. Much the same kind of results were obtained by Mothes (1926), who immersed the petioles of detached leaves in various nutrient solutions. Here again

[1] The term "sink" is here used in a sense analogous to that in which it is used in thermodynamics.

protein was hydrolysed in all but the youngest immature leaves. Similar experiments on tobacco leaves of unstated age carried out by Vickery *et al.* (1937) showed that a continuous digestion of protein occurred even under conditions in which carbohydrates were accumulating as a result of photosynthesis. It is possible that protein hydrolysis in these experiments of Pearsall & Billimoria, Mothes, and Vickery *et al.* is the result of severing, and Chibnall (1939) says "one is tempted to suggest that some influence of the root system, possibly hormonic, is responsible for the regulation of the protein level in leaves". Experiments of McCalla (1933) and Mothes (1931), however, suggest that protein synthesis can no longer occur even in attached leaves after a certain stage of growth. McCalla grew wheat in complete nutrient solutions up to the time of exsertion of inflorescences. Half the plants were then transferred to a culture solution without nitrogen and the remainder to a fresh supply of the original solution. The latter plants showed an increased absolute content of soluble organic nitrogen in the vegetative parts of the shoots, but the absolute protein content was unaffected, and fell at the same rate in both sets of plants. Here, then, even with the shoots still attached to the root system, and in the presence of an increased amount of soluble organic nitrogen in the shoots, proteins were progressively hydrolysed. In the kernels, which on the contrary are capable of active protein synthesis, the absolute amount of protein increased in the complete culture plants. Mothes carried out two experiments on *Phaseolus* and *Nicotiana* respectively. He chose leaves of different ages and removed the shoot immediately above them so as to direct the whole of the nutrient stream to the experimental leaf. The leaves were analysed at various times after "decapitation". The data show that, although absolute soluble nitrogen increased in certain cases, the increase led to further protein synthesis only in young leaves; in old leaves the protein-nitrogen content remained stationary. The results of all the above-mentioned experiments suggest that mature leaves are unable to synthesize protein. Contrary results were obtained by Engel (1929) on the attached first leaf of nitrogen-starved maize plants. When nitrogen was supplied to the roots, the protein content of the leaf increased during the following three weeks, whereas it fell in the case of untreated plants. No data are given to show whether the protein content of the leaf was already falling at the time of treatment, but we can suppose the leaf was mature, as no further increase in area occurred.

The experiment to be described in the present paper was carried out to determine whether there is an intrinsic factor superimposed on that of the external sinks in determining the net export of nitrogen from the mature and senescent leaf. An examination was made to discover whether protein synthesis could occur in a leaf after maturity, when the external sinks were removed as far as possible, and the supply of nitrogen was increased.

Experimental Procedure

Cape barley was set to germinate on 21 February 1939 (day 0), and on 23 February twelve seeds were planted out into each of 132 pots arranged in three blocks in the glasshouse. The pots contained a mixture of 3 kg. Waite Institute loam and 1 kg.

sand. The water content of the soil was maintained at 60% saturation throughout the experiment, and 200 g. of gravel were spread on the surface of each pot to reduce evaporation. On day 6 the plants were thinned so that six plants of 6–8 cm. in height remained in each pot.

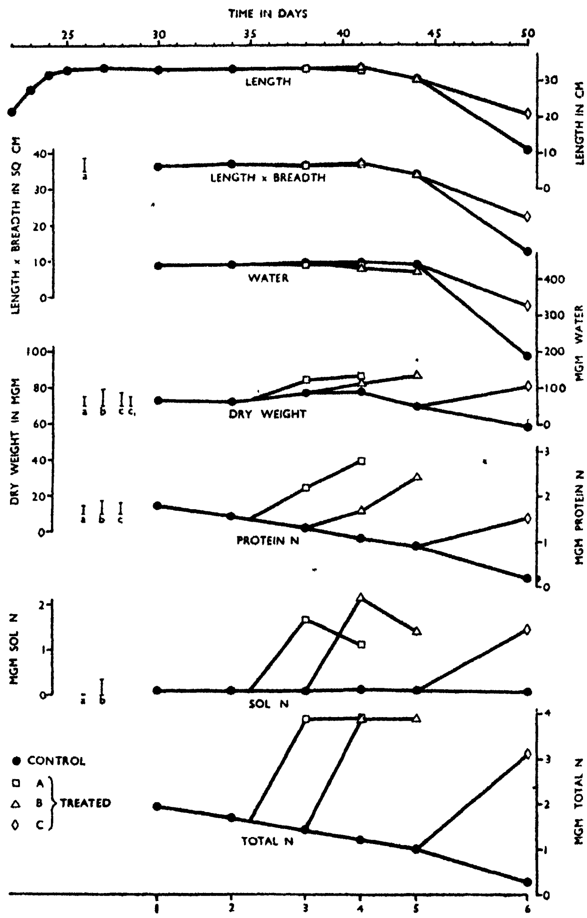

Fig. 1. Mean absolute values of various attributes of fourth leaf of barley. For each curve the significant differences at the probability level of $P=0{\cdot}01$ are as follows: *a*, sig. diff. between any black points; *b*, sig. diff. between any white points; *c*, sig. diff. between all points at the same harvest; c_1, same as *c* except that the probability level is $P=0{\cdot}05$.

The following nutrients were supplied to each pot: 0·15 g. $Ca(H_2PO_4)_2.H_2O$ on day 7; 0·15 g. $MgSO_4.7H_2O$ and 0·15 g. KCl on day 12; and 0·15 g. $Ca(H_2PO_4)_2.H_2O$ on day 17. No extra nitrogen was given until the time of treatment.

The fourth leaf of the main shoot was selected for investigation and, as this leaf approached its full size, daily measurements were made to ascertain when the maximum length was attained, this being accepted as a criterion of maturity. The first harvest was taken 5 days after the attainment of this stage. Thereafter length

and breadth of each leaf were measured at each harvest. The product of these measurements was considered an adequate index of area, as little variation was recorded during the experiment; moreover, Hopkins (1939) has shown that, for wheat leaves, there is a linear relationship between area and the product of length and breadth. Dead tissue was excluded from the samples in harvests 5 and 6.

Mean values for one leaf

Harvest	Day	Treatment	Colour	Dry wt. mg.	Abs. water mg.	L. × B. sq. cm.	Abs. protein N mg.	Abs. soluble N mg.
1	30	Control	GY $\frac{4}{6}$	73·4	440·4	36·36	1·82	0·12
2	34	Control	GY $\frac{4\cdot8}{4\cdot7}$	72·6	442·0	37·02	1·56	0·12
3	38	Control	GY $\frac{5}{5\cdot2}$	77·5	447·5	36·79	1·31	0·11
		A	GY $\frac{4\cdot6}{4\cdot8}$	84·4	442·3	36·59	2·19	1·68
4	41	Control	GY $\frac{5\cdot3}{7}$	78·0	450·2	37·25	1·07	0·14
		A	GY $\frac{4}{5\cdot3}$	86·9	440·9	36·92	2·79	1·11
		B	GY $\frac{4\cdot7}{6}$	82·5	430·9	37·44	1·67	2·16
5	44	Control	GY $\frac{5\cdot7}{7\cdot3}$ tending to YGY $\frac{7}{7}$	69·8	443·2	34·34	0·91	0·11
		B	GY $\frac{4\cdot3}{6}$	87·0	421·6	34·12	2·43	1·41
6	50	Control	Variable: Y $\frac{8}{6}$, YRY $\frac{8}{6}$, with patches of GY $\frac{6}{6}$	58·3	187·6	12·66	0·19	0·09
		C	GY $\frac{5\cdot2}{6\cdot2}$	81·1	326·8	22·11	1·53	1·59

During the afternoon preceding each harvest, the pots to be harvested were taken from the glasshouse and placed in cabinets maintaining a constant temperature of 24° C., and a light intensity of approximately 800 m.c. The humidity was controlled as far as possible. This equalized the environmental conditions during the 16 hr. prior to each harvest.

On day 35, eight pots from each block were selected at random and the whole of the main shoot above the ligule of the fourth leaf was removed, all the tillers being cut off as near soil level as possible. Thus only four leaves remained on each plant. Each of these pots was given at the same time 1 g. of ammonium sulphate in solution. These were designated A plants. On day 38, twenty-four more pots were treated in exactly the same way as the A plants. These were called B plants. A further set of

twelve pots, the C plants, were treated on day 44. At each harvest one replicate of four pots of control plants was taken from each block. At harvest 3 (day 38) half the A plants were taken in the same manner; at harvest 4 (day 41), the remaining A plants and half the B plants; at harvest 5 (day 44), the remaining B plants; and at harvest 6 (day 50), the C plants.

At the time of harvest the fourth leaf on each plant was severed at the ligule and measured. A number of these leaves were also chosen at random and the colour matched as nearly as possible with the standards contained in the *Munsell Book of Color* (1929). The twenty-four leaves were then bulked and chaffed. Two subsamples of approximately 3 g. were weighed and used for protein-nitrogen and total-soluble-nitrogen estimations according to the tungstic acid precipitation method given by Petrie & Wood (1938*a*). The remainder was divided into two parts which were weighed and then dried at 95° C. to constant weight for dry-weight estimations. Thus each recorded value represents the mean of duplicate analyses on three separate samples.

Discussion

Considering first the data obtained for the control plants, we find that only the dry weight showed an increase after maturity, this being significant between harvests 2 and 4. The total nitrogen and protein-nitrogen contents of the leaves fell continuously from harvest 1 onwards. Richards & Templeman (1936) also found that, after complete expansion, the fourth leaf of barley showed a decline in percentage nitrogen content. The increase in dry weight observed in the present case was probably due to the accumulation of carbohydrates and general increase in the mechanical tissues of the leaf. It is noteworthy that not until approximately half of the protein had been hydrolysed was there any fall in the absolute amount of water in the leaf.

During the whole of the experimental period there was no significant decrease in the amount of soluble nitrogen in the leaf with time. The question arises as to whether this phenomenon is compatible with a hypothesis that net export is wholly the result of the presence of other parts of the plant acting as sinks. The sinks must act by causing translocation of nitrogen from the leaf to exceed that of import of nitrogen from the external medium. Let us suppose that a steady state can exist in the leaf between the amount of proteins and that of amino acids, such that the former increases with the latter, the water content remaining unchanged as was the case in this experiment (Petrie & Wood, 1938*a*, *b*); it is then clear that, if amino-acids are continuously removed by translocation, there will be a continuous protein hydrolysis, accompanied by a continuous fall in the amount of amino-acids. That such a fall occurred is not precluded by the data if the ratio of amino-acid nitrogen to that of total soluble nitrogen decreased in such a way that the latter remained constant.[1] The amount of amino-acids themselves, however, could have remained constant if one or other of two states of affairs existed. The first of these is that there was an intrinsic change in the steady state relationship such that, for a given amount of

[1] For this there is admittedly no evidence in this experiment, though Smirnow's data for sunflowers show a decrease in proportion of amino-acids in the leaves as a whole (1928, p. 728).

amino-acids, there was, at the steady state, a smaller amount of proteins; this state of affairs could result from a decreased rate of synthesis or an increased rate of hydrolysis. This would represent an intrinsic factor superimposed on that of the sinks as a determinant of nitrogen export, and could have an effect of such magnitude as just to counterbalance the tendency of the amount of amino-acids present to fall with time. The second state of affairs is that there was no intrinsic change in the synthetic system; but on the other hand, there was a change in the proportions of individual amino-acids, due to differential breakdown or removal (Petrie & Wood, 1938*b*). If one or more of the amino-acids were undergoing such preferential removal, protein hydrolysis would cause the others to accumulate, so that it is again just possible for no appreciable change to occur with time in the total amino-acid content. Finally, what seems more probable than the previous suppositions, is that the curve relating proteins to amino-acids at the steady state is very steep, in which case the fall in the amino-acid concentration with time might have indistinguishably affected the amount of soluble nitrogen in the leaf in the present experiment. These considerations are put forward to show that, although the constancy of total soluble nitrogen is of considerable interest in the present connexion, it does not in itself establish the existence of an intrinsic factor leading to an acceleration of protein hydrolysis in the leaf with age.

When the A and B plants were treated we find, then, that the protein content of the leaf was steadily falling. It is obvious from Fig. 1 that harvest 4 marks a definite stage in the history of the control plants, for both the dry weight and area begin to diminish. These changes we may regard as indicating the onset of senescence. Thus by day 44, when the C plants were treated, the leaf was definitely senescent and by harvest 6 only a small part of each leaf of the controls was still living.

The effect of treatment was both striking and immediate on all three sets of plants. Of these, the A and B plants will be considered together. At the end of 3 days, the increase in protein-nitrogen content was very marked, and the soluble nitrogen rose to more than ten times its original value. During the 4th, 5th and 6th days of treatment, the protein-nitrogen content continued to rise but the soluble-nitrogen content fell. The total, however, remained constant in both sets of plants. It appears, therefore, that a direct conversion of soluble nitrogen into protein nitrogen took place during this period. It is also apparent that soluble nitrogen accumulated in the leaf much more rapidly than it could be converted into protein. The initial rapid increase in soluble-nitrogen content may have been largely in the form of inorganic nitrogen, the conversion of this into organic forms and protein being governed by the soluble carbohydrate concentration.

The C plants also showed a marked synthesis of protein, although treatment was delayed until the leaf was already senescent. Only one value was obtained 6 days after treatment, but it is possible that an initial increase in soluble nitrogen was followed by a decline in the same way as in the A and B plants.

No significant change in area or water content accompanied the sudden increase in metabolic activity in the A and B plants. Ruge (1937) found that the intermicellar portion of the walls of parenchymatous cells undergoes a change with age from a

water-soluble pectin which is elastic to an insoluble form incapable of stretching. It is obvious that a sudden renewal of synthetic activity in the leaf is not accompanied by a reversion of the cell walls to the elastic state. This fact may account for the lack of increase in water content, which would be limited by the size of the cells. That water content and area are closely related has also been shown by Richards (1932) and Watson (1939). No direct relationship appeared to exist between the water and nitrogen contents.

The decrease in area in the C plants between harvest 5 and 6 is due to the fact that, at the time of treatment, the leaves were pale yellow-green grading to pure yellow near the tip, the tip itself being dead; and during the 6 days after treatment the portions of the leaves which were quite yellow died, so that this part was not included in harvest 6. The rest of the leaf became noticeably darker green. During the same period, however, the greater part of each leaf in the controls died; so that we can conclude here that treatment arrested senescence.

There was a significant increase in dry weight in all the treated plants. This could be largely accounted for if the total increase in nitrogen content represented an increase in organically combined nitrogen. The increase in chlorophyll content, as judged by the colour change, may also have resulted in a greater production of carbohydrates.

The supposition that leaves completely lose the power of protein synthesis at maturity has thus been wholly discounted in the present experiment with barley. It was apparent, however, that the rate of protein synthesis, whether expressed as grammes per unit area, or grammes per gramme protein nitrogen, declined with age. This may have been due to decreasing carbohydrate content of the leaf, causing a decreased rate of formation of organic nitrogen compounds. On the other hand, it may indicate that there is an intrinsic shift with age in the rates of protein synthesis or hydrolysis of proteins. The stage at which the leaf entirely loses the capacity for protein synthesis is not known; but from observations on the present experiment it seems probable that in barley it is retained so long as chlorophyll is present.

These results are thus at variance with those described earlier in the paper, and therefore provide further evidence for believing that the behaviour of excised leaves is abnormal. It is more difficult to see why they disagree with the results of McCalla and Mothes on attached leaves. In McCalla's case it must, however, be admitted that it was not shown that the soluble organic nitrogen compounds increased in the leaves themselves. Soluble organic nitrogen may have been synthesized in the roots and then have been carried up the stem directly to the inflorescence.

The tissues in a leaf are not all of equal age. Monocotyledonous leaves develop from a basal meristem (Arber, 1934, p. 47), so that there is a gradient from young to senescent cells from the base towards the tip. Avery (1933, p. 569) has shown that the same kind of gradients exist in tobacco leaves. There is no reason to suppose therefore that dicotyledonous leaves under the same conditions as the present experiment would not exhibit the same phenomena as barley leaves.

The data of this experiment can thus be satisfactorily explained on the assumption that net export of nitrogen from leaves is mainly due to the presence of sinks,

which causes an increased rate of translocation of soluble nitrogen compounds. Mature and senescent leaves do not lose the capacity for synthesizing protein. It is possible, however, that the rate at which protein can be synthesized declines progressively with age, and this factor may be superimposed on that of the sinks in causing net export.

SUMMARY

Experiments carried out by various workers with detached foliage leaves in nutrient solutions, and with "decapitated" plants, suggested that the mature leaves of annual plants were unable to synthesize protein.

In the present experiment the fourth leaf of barley was investigated at three different ages after it had reached its maximum area. All the tillers and the main shoot above the ligule of the fourth leaf, which acted as sinks, were removed, and extra nitrogen was supplied to the roots. Under these conditions protein nitrogen was rapidly synthesized in the leaves in each of the three sets of leaves examined. The capacity for synthesis was apparently retained so long as chlorophyll was present, but the rate of synthesis declined with age. Whether this was due to an intrinsic change in the leaf or was due to some other cause is not known. This factor may be superimposed on that of the sinks. The latter must however be regarded as the main factor causing net loss of protein from mature leaves.

The writer desires to acknowledge her indebtedness to the University of Adelaide for permission to carry out this work at the Waite Institute; to Dr A. H. K. Petrie for his help in planning the experiment and in the preparation of the manuscript; and to Mr E. A. Cornish for help in the statistical analysis of the data.

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POLLEN ANALYSIS AND FOREST HISTORY OF ENGLAND AND WALES[1]

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(With 13 figures in the text)

CONTENTS

INTRODUCTION

In the period which has passed since the last retreat of the ice sheets from Britain, changes of many kinds have taken place in our country, some leaving their clear traces, others faint record or none. Successive vegetation types have occupied the ground, forests have become established and the dominant trees in them have changed and extended or retrogressed in importance. Different species of plants and animals have appeared, shifted their range, and some have vanished. Different races of men have migrated across the face of the country, and customs and cultures have diffused from one race to another. Lakes have become filled with muds or silts, and plant invasion has affected them to varying degrees, their sediments altering with changing climatic drift and changing topography. Rivers have changed their volume and course and peat has grown in fens and bogs. On our coasts sand-spits and bars have formed, and salt marshes have developed behind them; estuaries have been invaded by the sea, and open land connexion with Europe has been severed. Although all these phenomena can be separately investigated, pollen analysis is perhaps the easiest single approach to the wide field of post-glacial change in which they all lie. It is not claimed, however, that it gives results of greater validity than do other methods, and it is clear that results from all fields must be successfully combined in any account of post-glacial events which is to have lasting value.

[1] Paper read before the Linnean Society of London, 14 March 1940.

In 1928 Gunnar Erdtman summarized the results of his own preliminary investigations of post-glacial forest history in the British Isles. Stimulated by his visits to this country and his writings, which for the first time effectively brought the method developed by Lagerheim and von Post to the notice of English-speaking people, a considerable body of work has been undertaken, though the number of workers is still very small in comparison with the interest and size of the field for inquiry which the peculiar conditions of this country present. The following paper will aim at answering two questions. Following the lead of this earlier work, how far has it now proved possible to produce a convincing scheme of post-glacial forest history for this country, and to what extent has it been possible to produce for it an independent correlation of this history with post-glacial events of historical, geological, archaeological, botanical and climatological character?

The first of these questions demands to know the intrinsic completeness and range of our present knowledge of forest history: the second, the extent to which this knowledge has been successfully linked to other fields of knowledge.

The free state of Eire has had the foresight and fortune to employ, in its uniquely promising country, Dr Knud Jessen, and he has trained able students who maintain his standards in their investigations. So far, comparatively few results have been published and, as little has also been produced from Scotland, in the meantime this survey must be limited to work from England and Wales.

Extension and revertence of woodlands

Since our most detailed diagrams have come from the Fenland of East Anglia, it will be convenient to employ the zonation which has been developed for that region and attempt to extend it to studies farther afield.

The longest diagrams show certain very striking features in common (see Fig. 1). Each shows a threefold divisibility into phases which reflect increasing warmth, maximum warmth, and diminishing warmth, but at the same time the movement of the curves in the third phase is much less than that in the first and must reflect much less severe climatic change. The five common genera of trees—birch, pine, elm, oak and lime—show these changes most clearly. Birch, the least exigent of them, has a maximum at the beginning, but its curve is strongly concave in the middle phase, and recovers a little in the third phase. The pine shows a maximum after that of the birch, but also has a concave curve in the middle phase, showing a little or no recovery in the last phase. Elm, oak and lime, which are trees demanding greater warmth, naturally show convex curves. They appear in order of their warmth requirements, viz. elm, oak and then lime. Of the three the lime is the tree most restricted in Britain by its warmth requirements, and it evidently persisted in substantial proportion only throughout the middle phase of greater warmth. Elm probably also diminished in the third period.

In this way it is clear that the principle of "revertence" expressed by von Post is quite applicable here. We should note further that the curves of the warmth-demanding trees (as well as hazel) begin their ascent suddenly and with vigour. The transition from the middle to the last phase is, by contrast, gradual, the curves

gently dying away. This gives the whole set of curves a strong lop-sidedness, or asymmetry about the middle period. The explanation of this may be that the warmth optimum was reached rapidly and that the following decline was much slower, but it may alternatively relate to conditions of spread of new trees into regions for the first time made available to their colonization, in contrast with those after establishment of closed forest.

So far we have considered the behaviour of the tree-pollen curves to be fairly attributable to temperature change, but we must now add comment on the alder curve, which suddenly rises from very low values to strikingly high ones, which are

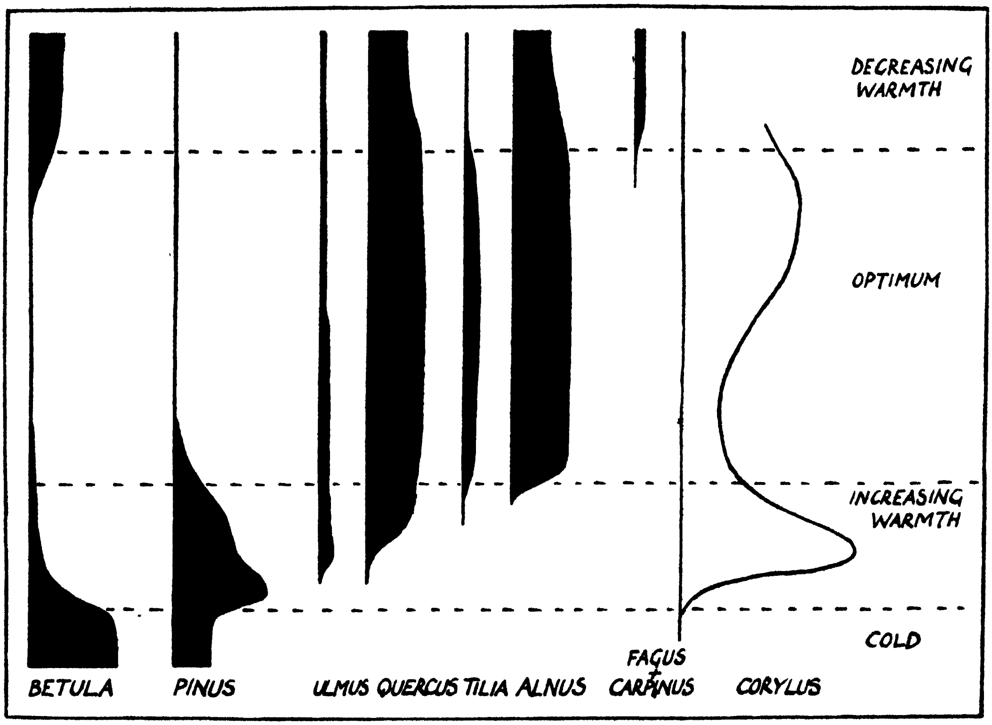


Fig. 1. Generalized form of pollen diagram of the East Anglian type to show the threefold divisibility of the post-glacial period, and the revertence in the last phase.

maintained throughout subsequent forest history. The presence of this species in small amounts long before this rise and over wide areas shows that the tree must have been present already. The only factor likely to have caused such sudden expansion of alder, and not of the other forest trees, is that of suddenly increasing wetness, and it may quite conceivably be related to the formation or sudden extension of the North Sea.

The third phase of the diagram shows a locally continuous beech-pollen curve and less frequent hornbeam, although scattered pollen of these trees has been present earlier. It has never been plausibly suggested what climatic change the spread of the beech reflects. It can hardly be diminishing temperature, since the

tree appears to be restricted in its natural westwards extension in this country by late frosts and by low summer temperatures determining flower formation. Nor at first sight is increasing moisture a plausible explanation, since the tree avoids the wet western part of the country. If we were to take the present natural distribution of beech and hornbeam in Britain as our criterion we should recognize a distribution centred in the south and east, and should have to postulate for the Sub-Atlantic period a climatic change towards increasing warmth and continentality. This is far from what other lines of evidence suggest.

It is possible to advance a conjecture, however, that the chalk and limestone soils now mostly occupied by natural beechwoods in Britain were too dry to carry closed woodland in the Sub-Boreal period. Archaeological evidence tends to support this view. Only when these soils became moist enough in the Sub-Atlantic was large-scale extension of beech possible. It then invaded open land freely, although it had not been able to invade the established mixed-oak forests of earlier periods. At least equally puzzling is the behaviour of the hazel-pollen curve. It is true that the generally high values for hazel in this country compared with those on the continent agree with our experience that in moving across the British Isles into the atlantic climate of the west, hazel increases strikingly in abundance. At the same time, from site to site the hazel-pollen curves differ enormously in absolute magnitude and in the incidence of maxima and minima. Some consistency of behaviour is recognizable in the Boreal period where huge maxima are generally present, but after this time the movements of the hazel-pollen curves await both analysis and explanation.

It is worth while noticing that the revertence which our diagrams consistently show is expressed essentially by a return of the birch in importance: the stages of Boreal forest development are by no means simply retraced, pine shows no widespread prevalence nor do elm and oak disappear as one would expect if the whole story were one of simple climatic retrogression that merely reversed the stages of advance. In the last stage also beech and hornbeam have extended though absent before the warmth optimum. It is well to recall that the extension of successive waves of woodland types over land previously treeless would by no means necessarily indicate the changes to be expected with reversal of the climatic drift affecting an established forest cover. It becomes clear therefore that though the threefold climatic division of the post-glacial holds, this is a first analysis only, and considerable complexity underlies it.

Zonation of pollen diagrams

Comparison of pollen diagrams from England and Wales has suggested the practicability of applying to them a common zonation system already worked out for the East Anglian Fenland (Godwin, 1940*a*). We assume that deposits corresponding to the Upper and Lower Dryas clays and the intervening Allerød layers will be recovered here as in Ireland and Denmark, and therefore leave (following the scheme of Jessen) zones I, II and III to represent them, and begin our zonation with zone IV. This can be considered the opening of the "post-glacial" period after the

"late glacial". For details of the zonation in East Anglia the reader is referred to the paper above cited, but the outline characters of the zones are as follows:

IV. *Birch-pine zone.* This is a zone of dominance of *Betula* pollen, with *Pinus* as the only other important tree. Smaller amounts of *Salix* pollen are present, and in the south and east of Britain small quantities of the warmth-loving trees; small amounts of *Corylus* also occur in some sites, but the ratio of non-tree pollen to tree pollen is so high as to suggest an open landscape, over which these warmth-indicating pollens may have been carried considerable distances.

V. *Pine zone.* This zone in East Anglia seems to show general extension of forest, and in it there is a widespread replacement of *Betula* by *Pinus* as the dominant tree pollen. *Corylus* begins to extend very rapidly, and here and there small amounts of warmth-loving trees are represented.

VI. *Pine-hazel zone.* Zone VI is clearly divisible into three subzones, *a*, *b* and *c*, which Dr and Mrs Clapham have recognized already in Berkshire (Clapham, 1939), and Mrs Megaw (Hardy, 1939) in Shropshire. In subzone VI*a*, *Ulmus* extends swiftly, and *Quercus* though present is less important. The dominant pollen remains *Pinus* in the south-east of England and locally elsewhere, and *Corylus* reaches extremely high values.

Subzone VI*b* differs from the preceding simply in that *Quercus* pollen equals or exceeds that of *Ulmus*: *Corylus* remains exceedingly abundant.

In subzone VI*c* the East Anglian diagrams show the first tendency of *Tilia* and *Alnus* to extend, and *Corylus* is generally much lower than in VI*b* and VI*a*. *Pinus* sometimes shows retrogression before extending mixed-oak forest and *Alnus*, but prevalent dry conditions seem often to permit its local and temporary extension.

Zone VI must correspond with most of the Boreal climatic period of Blytt and Sernander.

Above zone VI alder and trees of the mixed-oak forest predominate in the pollen diagrams of the south and east of Britain, and further zoning must be rather tentative. We may, however, distinguish zones VII and VIII, with a transition zone VII–VIII between them.

VII. *Alder-oak-elm-lime zone.* The beginning of this zone is indicated by the sudden replacement of *Pinus* by *Alnus* as the most abundant tree pollen. In some places where *Betula* has been maintained in previous zones it may be this tree that recedes before the *Alnus*. *Tilia* expands considerably and its pollen in East Anglia reaches consistently about 5–10% of the total tree pollen. *Quercus* maintains the values of the previous period or may show some increase. *Ulmus* shows no decrease to begin with, but a decline half-way through zone VII seems to be widespread, especially in the west, and can be used as an index for a tentative subdivision of zone VII into subzones *a* and *b*.[1] Although the *Corylus*-pollen curve is so very variable it seems generally true that it is low in subzone *a* and rises considerably in *b*. Small amounts of *Fagus* pollen occur sporadically at different sites in this zone but have no diagnostic value for it.

VIII. *Alder-oak-elm-birch (beech) zone.* As mentioned earlier, there may be

[1] These subzones do not correspond with the zones *a*, *b* used in the Fenland paper.

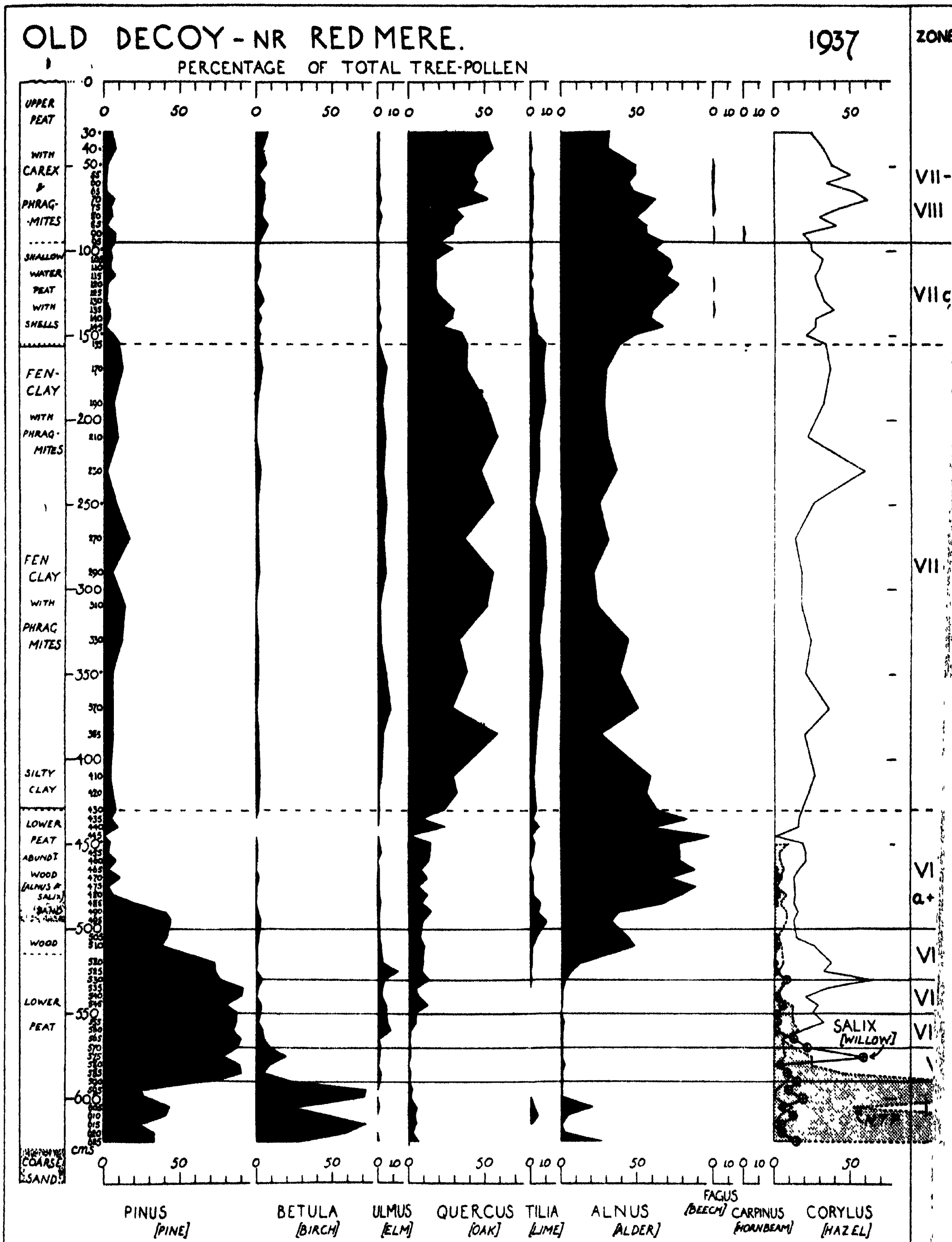


Fig. 2. Pollen diagram from Old Decoy, near Shippea Hill (reproduced from *Philos. Trans.*). The subdivision zone VII are different here, on account of the local conditions of Fenland, from those used in the rest of this pa N.T.P. is the total non-tree pollen expressed as a percentage of the total tree-pollen.

distinguished between zones VII and VIII a transition zone VII–VIII, and this has been recognized in the Fenland and Shropshire studies. There is not yet, however, evidence enough to warrant its employment in this general consideration of England and Wales as a whole, and it will suffice to say that in it the pollen curves change from the proportions of the earlier zone VII to those of the later zone VIII.

Zone VIII is characterized by three principal features: the *Betula* curve maintains substantially higher values than in the earlier zone, the *Tilia* curve either ceases or becomes discontinuous, and the *Fagus* (and to a less extent *Carpinus*) pollen is present in much greater amount than previously, in many sites continuously present although at low values.

PARALLELISM OF FOREST HISTORY ACROSS THE COUNTRY

The manner in which the zones above proposed can be applied to other parts of England and Wales may be seen by examining the diagrams from Cothill, Berkshire (Clapham, A. R. & B. N. 1939), from Shropshire and Flint Maelor (Hardy, 1939), and Tregaron (Godwin & Mitchell, 1938, where an earlier local zoning will be found to correspond substantially with the present zoning). With rather less clarity the zonation may be recognized in parts at least of many earlier published diagrams, where, however, the wide spacing of samples makes for uncertainty.

In order to determine more closely the applicability of the zone system to England and Wales as a whole, we have analysed all the diagrams already published for this area, together with several unpublished ones of our own from Norfolk, Somerset, Devonshire and Cardiganshire, and unpublished diagrams of other workers from Lindow Moss, Cheshire; from the Cornish valleys and from Ffos-Ton-Cenglau, Glamorganshire. We are extremely grateful to Dr K. Blackburn, Mrs E. M. Megaw and Mr H. A. Hyde for putting their results from these last-mentioned sites at our disposal.

From this analysis figures of average pollen composition have been obtained for each zone at many sites scattered over England and Wales, although comparatively few sites have a complete history extending over all the zones. For each zone a map diagram has been prepared in which each site is represented by a "clock-face" where the sectors represented by different symbols are proportional in size to the different tree pollens there present. These map diagrams are Figs 4–11, and the key to the sites is given in Fig. 3. In some areas the sites of pollen series lie so close that only one or two can be represented out of a great many: this is particularly true of the Fenland, but also holds for Somerset, Cornwall, Shropshire and to a less extent in north of England sites. At these places there is a good local agreement between the diagrams, and the actual evidence of uniformity of forest evolution will therefore be to this extent stronger than the diagrams allow us to show.

So far as possible the influence of pronounced local factors causing predominance of one tree or another (such as *Alnus* in fen woods) has been avoided by discarding those diagrams or parts of diagrams showing such effects. Sites at high altitudes have been generally avoided, although this has not been altogether feasible in the north of England. It will be clear from this that there is a subjective factor

Based upon the Ordnance Survey Map with the sanction of the Controller of H.M. Stationery Office.

Fig. 3. Key to sites employed in succeeding Figs. 4–11, or referred to in the text.

present in choosing the sites to be represented, in deciding the limits of the zones and in the recognition and exclusion of local effects. If, however, a consistent picture emerges from our comparisons we may probably assume that this factor has not led to important error. Lastly, it may be noted that two sites from Ireland have been included to facilitate the general extension of our results.

It will prove most helpful to consider these distribution diagrams one by one. In discussing them it will be assumed that the pollen composition is a good guide to forest composition, and there is in fact reason to think the two are closely proportional to one another.

In the birch-pine zone, IV (Fig. 4), it will be seen that *Betula* is predominant over the whole country: in Southampton it is exceeded by *Pinus*, but this must be regarded as a local effect. *Pinus* in general is rather more frequent in the south and east, where also there are at some sites small amounts of pollen of the warmth-loving trees, which may, however, be due to long distance transport into rather treeless country. *Corylus* is absent or present only in small amount, although it is certainly the western sites which show most of it.

In the pine zone, V (Fig. 5), it is evident that a general change has come over the woodlands of the country by the great increase of *Pinus* at the expense of *Betula*. This has led to a strong dominance of *Pinus* in the southern and eastern part of the country, though in the north and west *Betula* retains its preponderance. Local exceptions are evident at Hockham Mere in the one region and Lindow Moss, Cheshire in the other. Small amounts of the mixed-oak-forest trees (*Ulmus* and *Quercus*) are present in many, but not all sites. *Corylus* shows a very striking increase from the previous period, but this is far more strongly shown in the northern and western sites just as in zone IV. No doubt the hazel was favoured then as now by the maritime conditions of the west, and the climatic gradient of increasing continentality towards the east must have been greater then since the North Sea was at this time still dry land (note the analysis from the Leman and Ower banks, from peat now in 19 fathoms of water).

The pine-hazel zone, VI, opens with the subzone VI*a* (Fig. 6), and the diagram shows at once that considerable and widespread changes separate it from zone V. *Betula* has diminished but remains preponderant or very important in the north and west, just as does *Pinus* in the south and east. *Ulmus* and *Quercus* have extended tremendously, the former reaching surprisingly large values in many lowland sites: only in the north do these trees appear to have spread little. The extension of *Corylus*, although very variable from site to site, is very pronounced over the whole country, and values for hazel pollen of more than 100% of the total tree pollen are common. High values are not now characteristic of the west, but occur over the whole country, and one might possibly think this related to the North Sea transgression taking place at this time. *Tilia* is absent and *Alnus* almost so, except for surprisingly large values in the Isle of Man, no doubt the result of some local circumstance. It will also be noted that Hockham Mere and Lindow Moss still keep their differences from the regional trend, the one tending to show *Betula* instead of *Pinus*, the other the converse (see comment above).

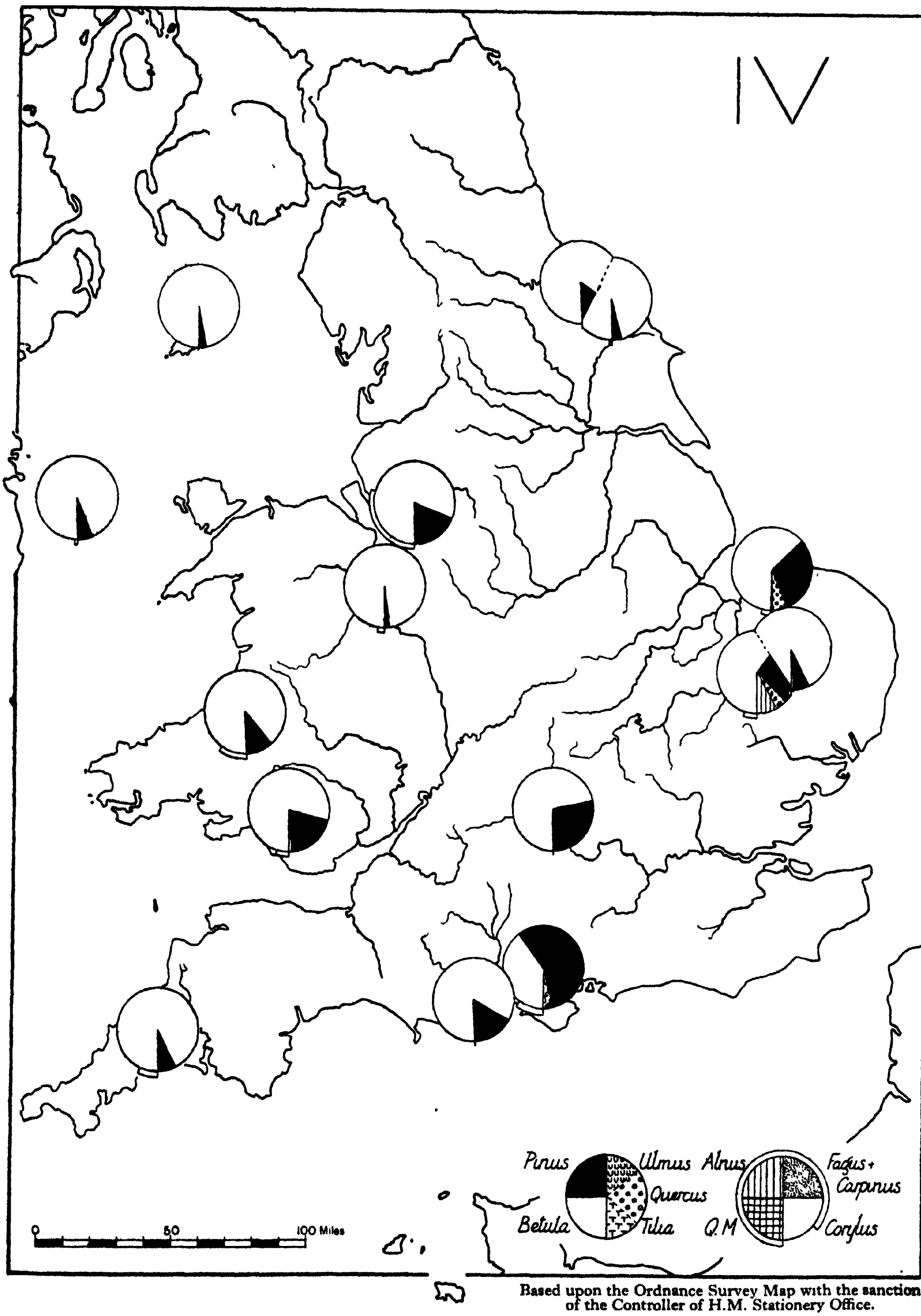
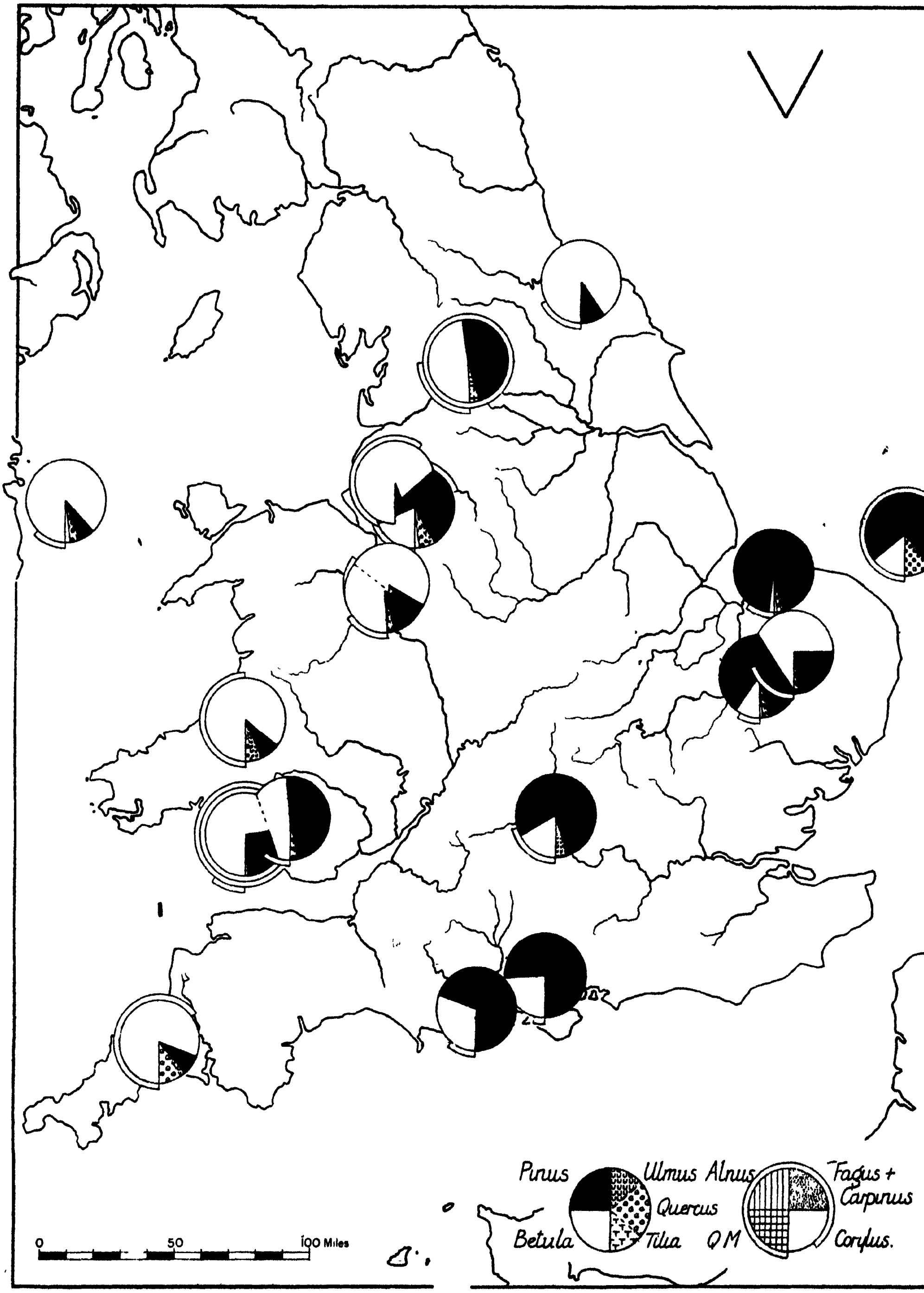
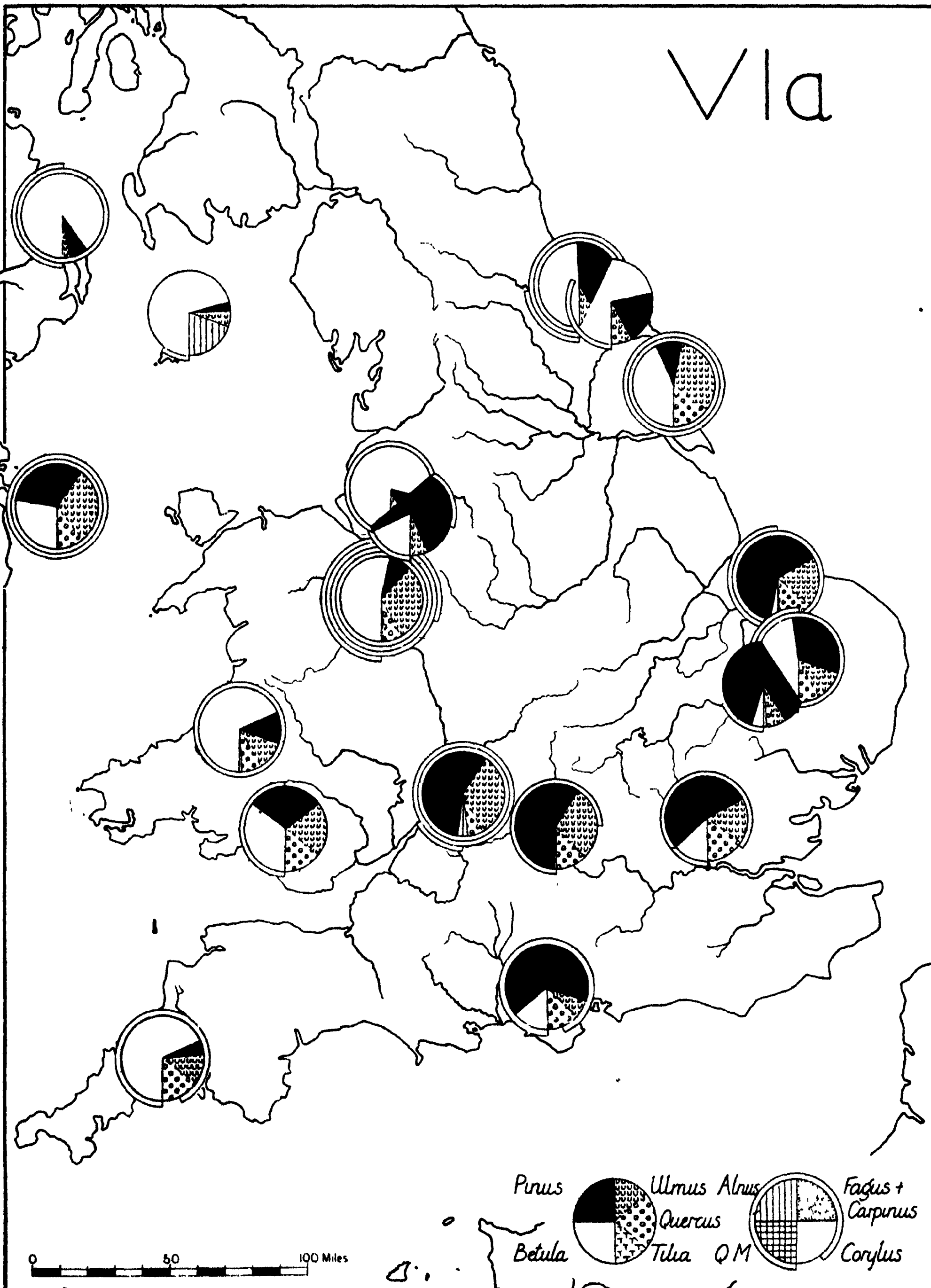


Fig. 4. Birch-pine zone distribution diagram. The size of each sector in the circles is proportional to the percentage of the tree pollen which it represents.



Based upon the Ordnance Survey Map with the sanction of the Controller of H.M. Stationery Office.

Fig. 5. Pine zone distribution diagram.



Based upon the Ordnance Survey Map with the sanction of the Controller of H.M. Stationery Office.

Fig. 6. Subzone (*a*) of the pine-hazel zone.

In subzone VI*b* (Fig. 7) the general aspect of the woodland has changed by the continued regression of *Betula*, and a continued expansion of *Ulmus* and *Quercus*, during which *Quercus* has become much the more prevalent. *Pinus* is of much the same status as the subzone VI*a*, and the *Corylus* has also changed little. In subzone VI*c* (Fig. 8) two new trees have appeared in substantial amount. The first is *Alnus* in all parts of the country, and the second *Tilia*, which is much more evident in the south and east than elsewhere. On the whole *Pinus* and *Betula* have diminished before the extension of Quercetum mixtum and *Alnus*, but at some sites *Pinus* has increased. Broadly speaking *Corylus* certainly shows far lower values than those of VI*b*: this is specially true of the west, but at a few sites there is some increase.

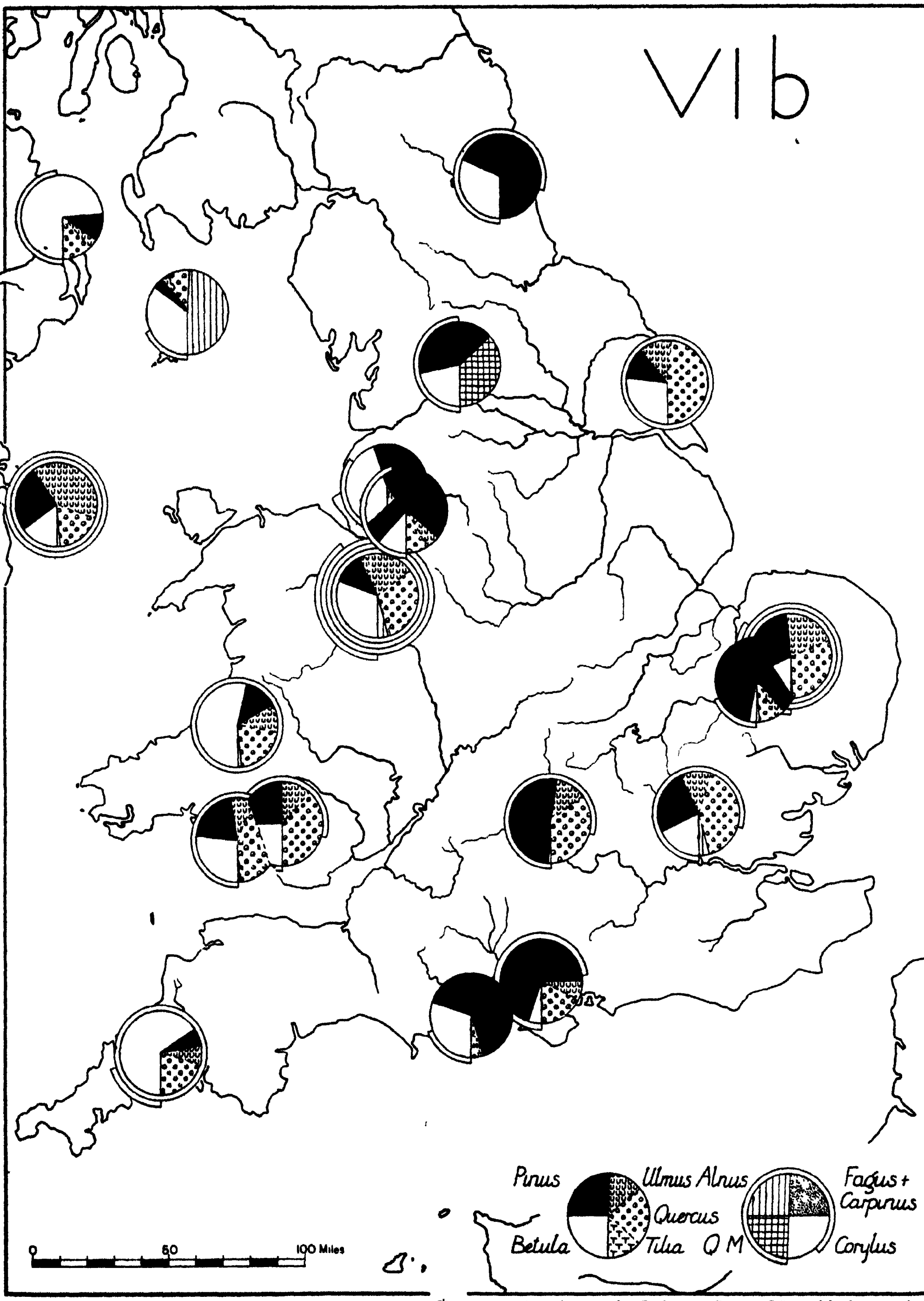
The alder-oak-elm-lime zone, VII, opens with the subzone VII*a* (Fig. 9), in which it is apparent that *Alnus* has suddenly become widespread over the whole of England and Wales: the values of more than 50% of some sites may be due to local over-representation, but it is abundant everywhere. This expansion of *Alnus* has taken place somewhat at the expense of the Quercetum mixtum, but it is particularly *Pinus* and *Betula* that have been displaced. Except for a few sites *Pinus* no longer reaches 10% of the total tree pollen. *Betula* has practically vanished from the east, is much reduced in Wales and the south-west, and retains substantial values only in north of England sites. These latter show such low values also for Quercetum mixtum as to support a general threefold regional differentiation of forest during this period. *Corylus* retains the generally low values of zone VI*c* and shows no regular variation across the country.

Subzone VII*b* (Fig. 10) differs in small degree only from subzone VII*a*, but it is interesting to see how consistently these small changes appear. Both *Betula* and *Pinus* have almost everywhere diminished, although at some sites in the north and extreme south-west *Betula* remains important or even dominant. *Alnus* and the Quercetum mixtum continue to be of similar importance, although *Ulmus* is now less a frequent component of the latter. It is interesting, as supporting the validity of distinguishing the two subzones, to find *Corylus* now much increased in importance, so that values of 100% of the total tree pollen are common, especially in the west and south-west.

We come lastly to consider Fig. 11, which represents the alder-oak-elm-birch-(beech) zone VIII. Consideration of this shows that all over the country *Betula* has greatly increased in importance again, so that, East Anglia alone excepted, it is of similar proportions in the forest cover to either *Alnus* or Quercetum mixtum. In the Quercetum mixtum, *Tilia* is now virtually absent save for a single site at Cardiff, which was similarly out of step in zone VII*b*. Here and there at scattered sites throughout the country *Fagus* plus *Carpinus* reach 1 or 2% of the total tree pollen, and we shall see that they are quite important in a few East Anglian sites. *Corylus* continues to be present in very variable but generally high amount.

As a result of this visual survey a number of conclusions of general interest may be drawn:

(1) The zones distinguished in the Fenland are applicable to the rest of England



Based upon the Ordnance Survey Map with the sanction of the Controller of H.M. Stationery Office.

Fig. 7. Subzone (*b*) of the pine-hazel zone.

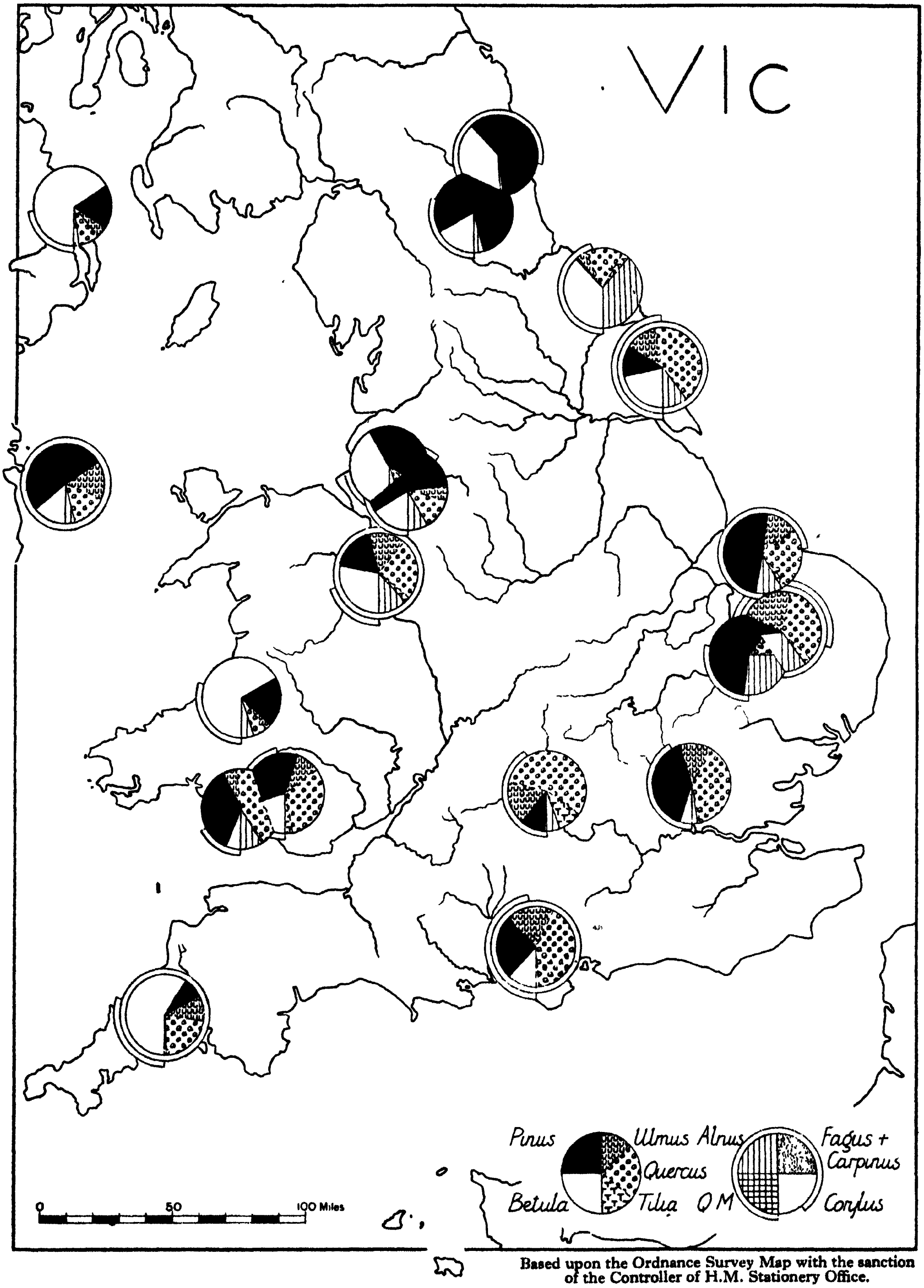
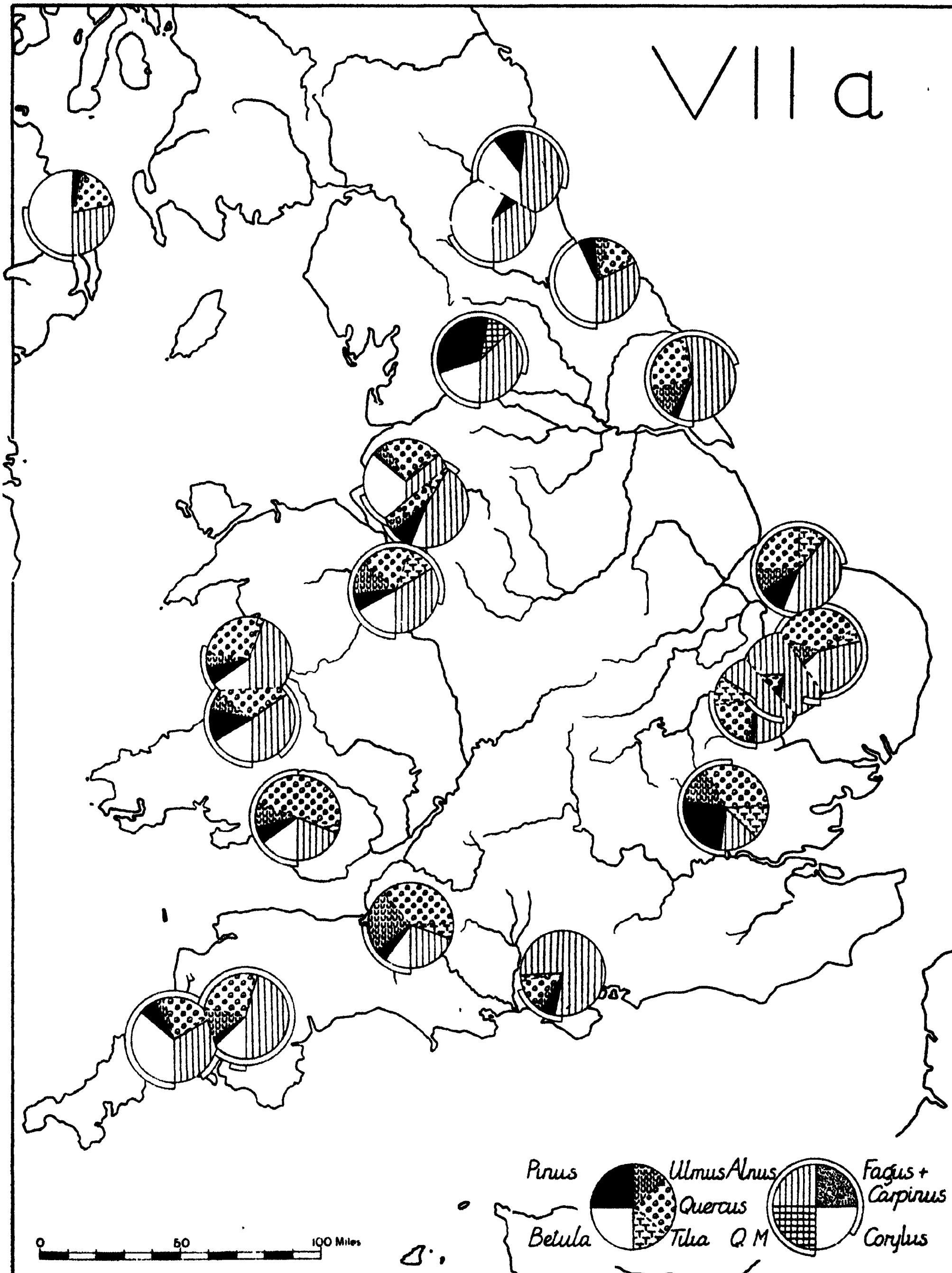


Fig. 8. Subzone (*c*) of the pine-hazel zone.



Based upon the Ordnance Survey Map with the sanction of the Controller of H.M. Stationery Office.

Fig. 9. Subzone (*a*) of the alder-oak-elm-lime zone.

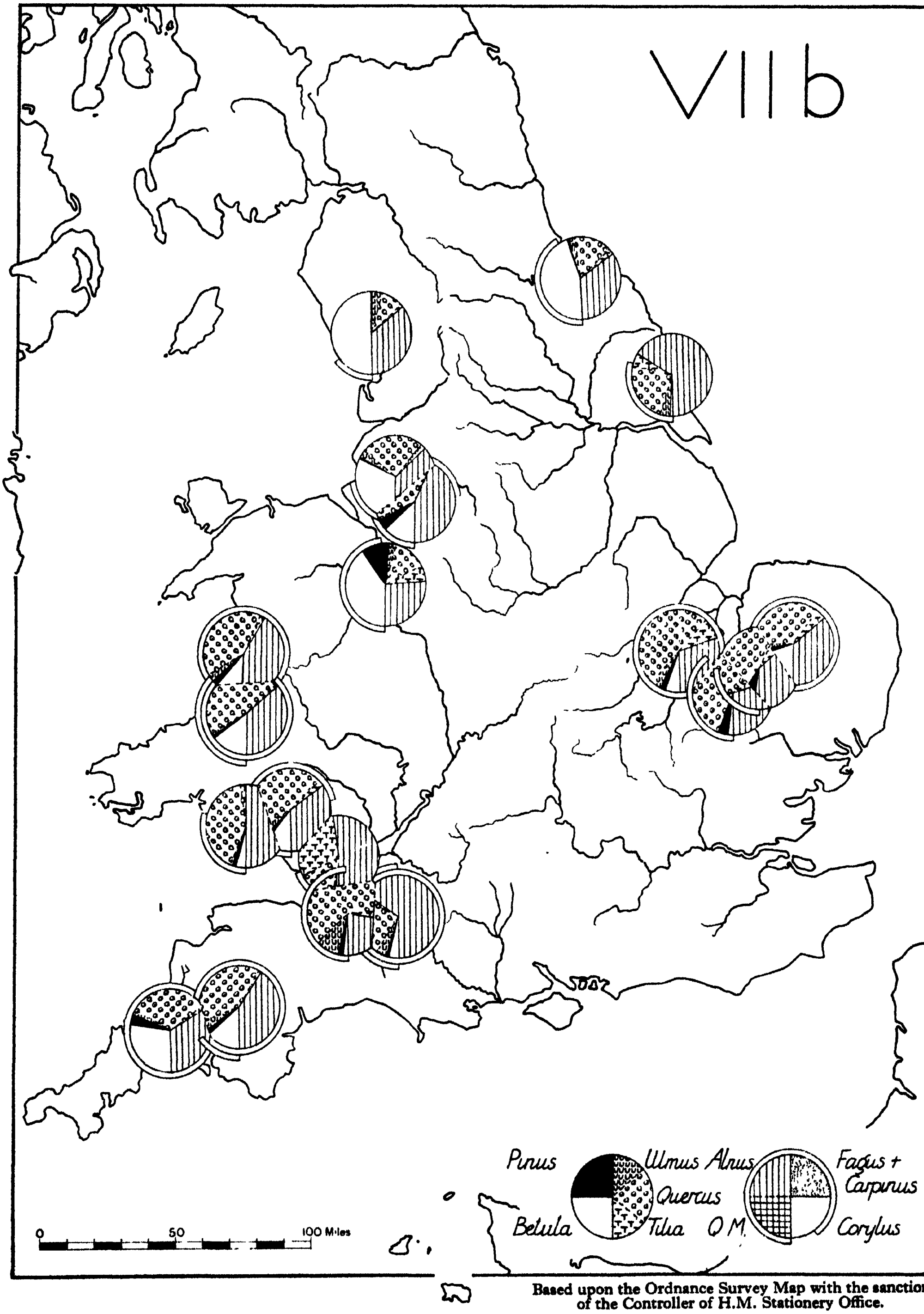


Fig. 10. Subzone (*b*) of the alder-oak-elm-lime zone.

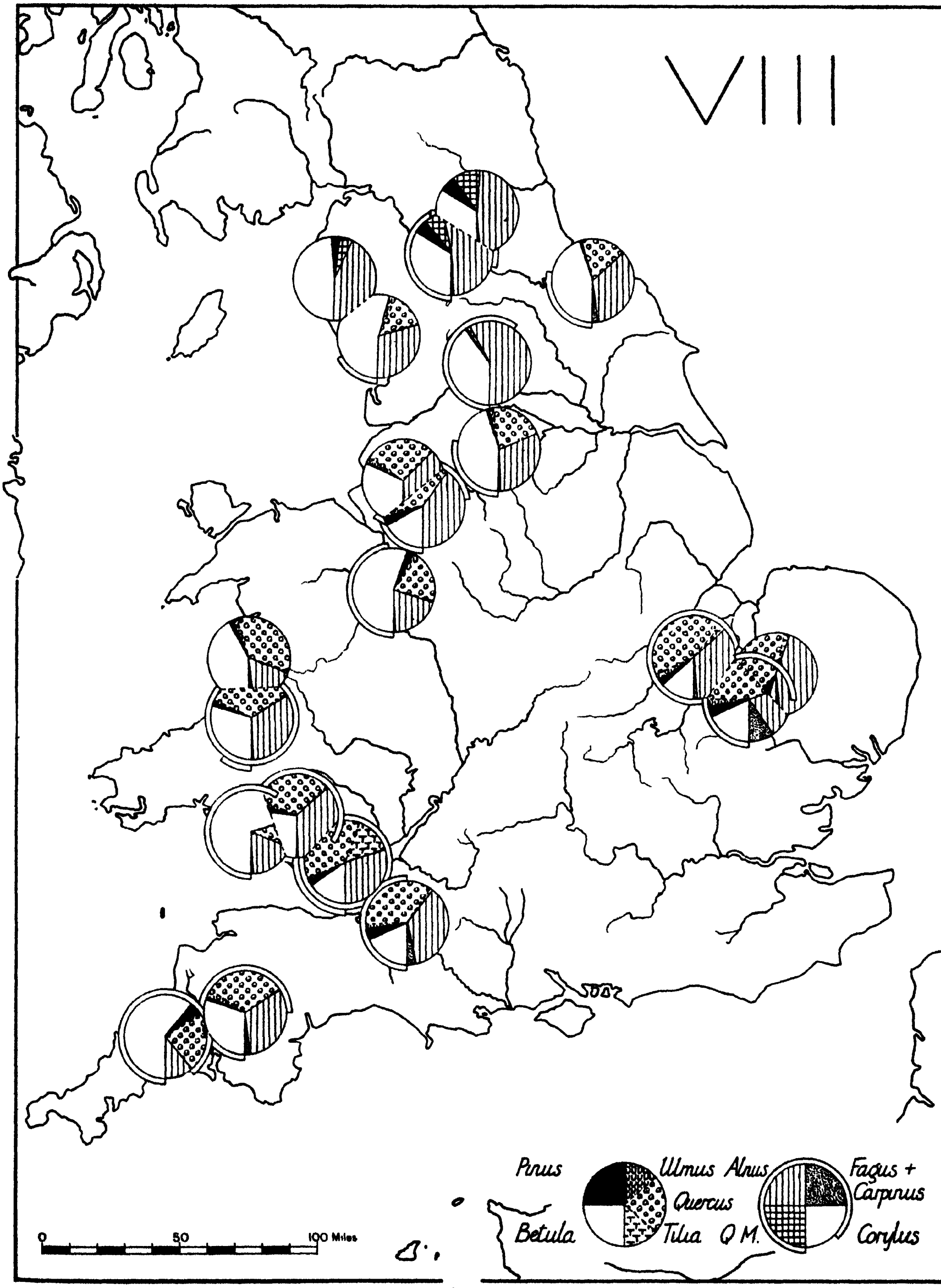


Fig. 11. Alder-oak-elm-birch-(beech) zone.

and Wales, and analysis based upon them gives at any one time a consistent picture of forest composition over the country as a whole.

(2) Despite the consistency above mentioned, in each successive period there is evident a similar regional differentiation of the country into a southern and eastern portion (roughly south-east of a line from Somerset to north Yorkshire) and a western and northerly region. Throughout the post-glacial period the south-east region has shown the greater development of warmth-loving trees, and the other has kept high values for less exigent species such as the birch. *Betula* values have tended to increase northwards, and *Corylus* values have been highest in the west, though this has been less evident after zone VI *a*. From his analyses of the British climate and its natural vegetation, Prof. Tansley (1939) has recently suggested that within this area there are three climatic-vegetational regions, *viz.*, the south-eastern, western, and northern. The boundaries of these in England and Wales correspond broadly with the regions made apparent by our own studies, and suggest the ancient and permanent character of this regional vegetational differentiation.

(3) It is therefore clear that the forest history of England and Wales shows the phenomenon of regional parallelism of development, to which attention was called by von Post (1929). Given climatic changes have induced *equivalent* but *not identical* changes in forest composition in different parts of the country throughout the post-glacial period. No doubt it will prove profitable in future work to distinguish two or three regions of England and Wales, devising for each a separate scheme of forest history. It is clear that such natural regions exist, but it is less clear how far they are due to climate, and how far to the factors of geology and altitude.

(4) Certain sites, such as Hockham Mere and Lindow Moss, have been already sited as "out of step": they appear so in every successive zone, and this must certainly be due to local factors (cf. Hesmer, 1933). The Hockham Mere site always shows more birch than neighbouring sites, and as it lies in the north of the sandy Breckland, we can suppose that the local soil conditions have always given its forests the features of pre-climax or edaphic climax. Lindow Moss on the other hand shows much less birch than neighbouring Chat Moss and nearby Pennine sites, and has had throughout a rather "post-climax" character. In the series of sites from Shropshire and Flint Maelor, the one we have chosen, Whattall Moss, has already been shown (Hardy, 1939) to differ steadily from the rest, almost certainly the result of local conditions in the area.

(5) The phenomenon of revertence, expressed by the spread of *Betula*, is seen to have been very widespread and to be a general feature of our forest history.

(6) The presence of small amounts of different trees in zones earlier than those in which their rapid extension takes place, suggests that the extension is due to climatic control and is not affected by the rate of natural spread of the trees.

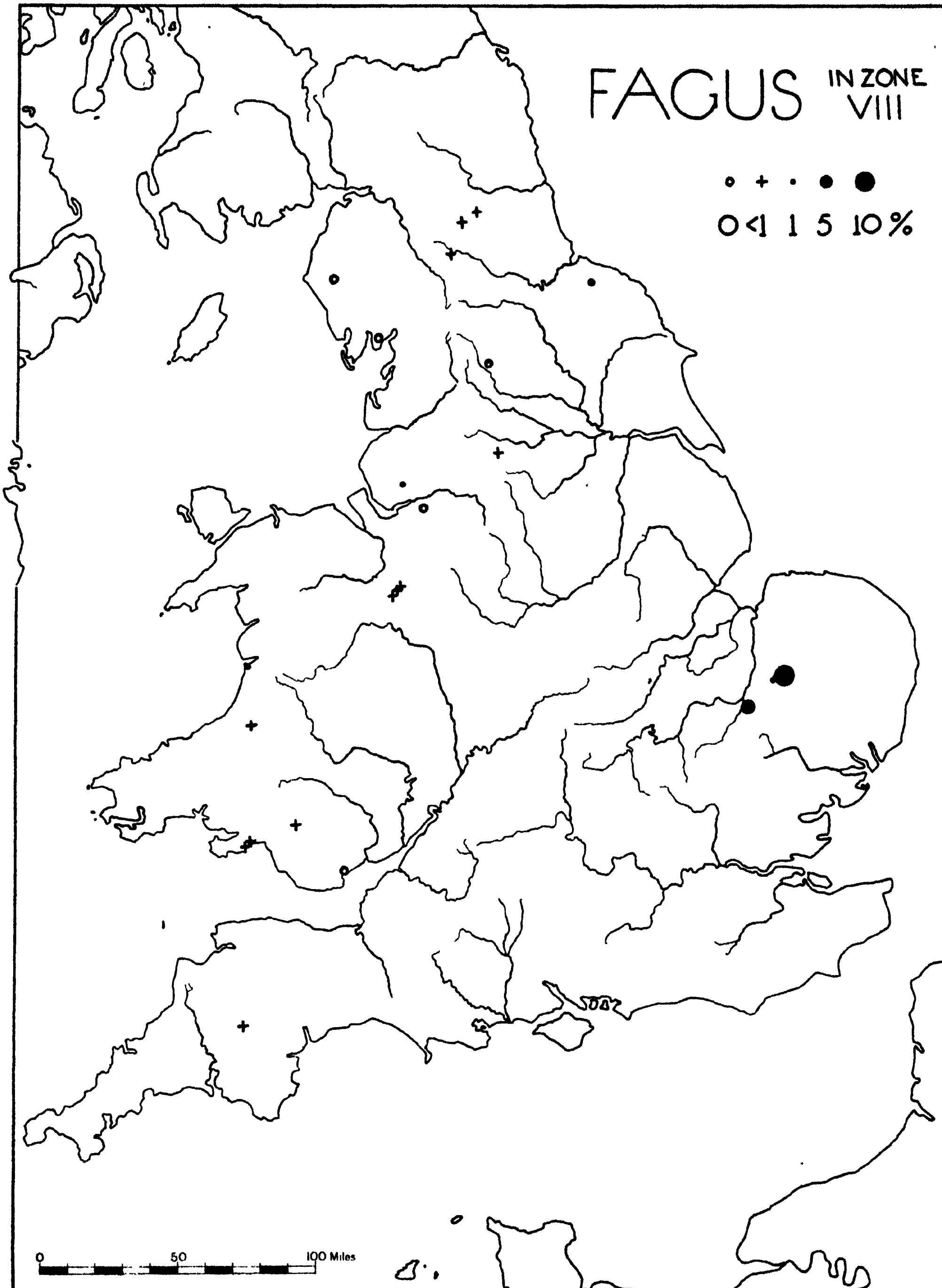
NATIVE STATUS OF THE TREES

From the analysis considered above it is perfectly clear that our familiar tree genera—*Betula*, *Pinus*, *Ulmus*, *Quercus*, *Tilia* and *Alnus*—must be indigenous, as also *Corylus*. In general terms there has been no doubt of this, although the problem is

less certain where species and not genera are concerned. Thus the pollen identifications do not allow (or have not in practice so far allowed) separation of *Betula pubescens* from *B. alba*, nor *Quercus robur* from *Q. sessiliflora* (although von Post has found the latter species separable in Scandinavia). Nor is it possible to say what species of *Ulmus* are represented, although the extension in zone VI*a* makes the question particularly interesting: it may well include *U. glabra*, but how far *U. stricta*, *U. procera*, *U. nitens*, or other species may be present, we have as yet no means of knowing. With *Alnus* and *Corylus* we are concerned with only one species in this country, and this is probably true also for *Pinus*. We cannot distinguish by the pollen the variety *P. sylvestris* var. *Scotica*. Nevertheless our results show beyond doubt what has often been contested, the truly native status of the pine in both England and Wales. In every zone and at almost every site pine pollen is recognizable in substantial amount, and in bogs still growing, pine pollen continues in small amount from the Boreal pine period to the present day, and there is no reason to doubt that the pine trees now growing on the bog surfaces are direct descendants of native trees.

With the genus *Tilia* more direct evidence is available, because the grains of *T. cordata* and *T. platyphyllos* are morphologically quite distinct. Although both species now regenerate in this country and appear to behave as native trees, grains of *T. platyphyllos* have never yet been reported. Many thousands of *Tilia* grains have been examined by the analyses made in the Cambridge Botany School alone, and they have all been unmistakably *T. cordata*, although the sites examined included some in the south-west not far from the present site of living *T. platyphyllos* at Symond's Yat. Further analyses at sites close to the present stations of native *Tilia* would be of very great interest, and autecological studies of both species of linden would be very helpful in interpreting the advance and regression of *Tilia* already described as happening through the climatic optimum.

The work we have been reviewing gives us no data for assessing the status of species of *Salix*, *Populus*, *Acer*, *Taxus* and other genera, for either the pollen of these genera is not preserved or the different species have not hitherto been separable by pollen characters. Two species however remain for special consideration: these are *Fagus sylvatica* and *Carpinus betulus*. The native status of the beech has long been denied or doubted. It has been held that the tree was introduced by the Romans, but by now a long list of pollen diagrams has shown small amounts of *Fagus* pollen at levels as old at least as the Bronze Age, and Hyde (1937), has recently reported the *wood* of the tree in charcoal from an Iron Age site in South Wales. Fig. 12 shows the average values for beech pollen, throughout zone VIII, and it will be seen that during this period amounts of this pollen from 1 to 10% were present at many sites in England and Wales, and smaller amounts were recognized widely. At few places was the pollen of the beech entirely absent, but the highest values have come from the eastern margin of the Fenland, where the peat deposits doubtless caught the pollen rain from trees growing on the chalk ridges nearby. At sites such as Wicken Fen and Wilton Bridge, *Fagus* and *Carpinus* are clearly important components of native woodland throughout zone VIII (Godwin, 1940*a*), though present



Based upon the Ordnance Survey Map with the sanction of the Controller of H.M. Stationery Office.

Fig. 12. Distribution map to show average amounts of beech pollen in zone VIII at sites all over England and Wales.

sporadically already in zone VII. It would be of the greatest interest to trace the history of these trees by pollen series in sites well within the present distribution of beech and hornbeam woods in the south and east of England. Unfortunately growing peat bogs are rare in this region.

ARCHAEOLOGICAL CORRELATIONS

Through the active cooperation of members of the Fenland Research Committee it has proved possible to link forest history and human pre-history by investigations at a large number of Fenland sites, and to obtain correlation of our pollen zoning with cultures from the Mesolithic right through to the Romano-British. It is not proposed to recapitulate the details of this, but an outline of the results will be seen in the first column of Fig. 13 where they will serve as a base from which we can extend the correlation to other parts of Britain. Naturally not all the sites shown in this schema have an equal weight. Some occur in long closely spaced pollen series in an area where intensive studies have been made, others depend on few analyses in a region where the drift of the pollen curves is still not very well known. Several sites of relatively minor importance, but where some direct correlation has nevertheless been possible, have been omitted altogether: they include Lower Halstow (Burchill, 1925–8), Isle of Wight (Clifford, 1937), Southampton and Barry Docks (Godwin, 1940*b*; Godwin, H. & M. E. 1940). It is enough to say of such sites that results from them do not conflict with those presented here.

The Mesolithic site at Broxbourne may possibly be older than zone VI*c*, but cannot be younger. The Meare Lake Village site lies at the top of a series and it is possible that there is some discontinuity between the occupation level and the top peat samples: the same applies to the two late Roman hoards on Shapwick Moor, where, in each instance, the hoard was interred from a surface at least as high as the bog surface to-day. The correlation of the famous Llyn Fawr hoard with the pollen zones of the neighbouring site at Ffos-ton-Cenglau, rests a good deal on the indirect evidence of peat-stratigraphy. At Llangeitho and Whattall, though the level of the artifacts is fairly clear in relation to the pollen zones, the exact age of the object is not intrinsically evident. At Skipsea the pollen series were collected some years after discovery of the Maglemose fish-spear and some distance from the actual site of the find, so that although the spear clearly came from the lake muds, it might belong to any part of zone VI, or perhaps be even earlier.

Despite these weaknesses, inevitable in the absence of heavily financed and laborious excavations directed to our own special ends, these additional results for the rest of England and Wales are fairly consistent in themselves and in agreement with those from the Fenland. The Mesolithic is seen to lie in zones V and VI, although the Mesolithic at Lower Halstow on the Essex coast and in the Isle of Wight extends into zone VII. The Neolithic lies in the middle of zone VII. Romano-British sites clearly fall into zone VIII. The transition zone VII–VIII seems to take in various cultures from middle Bronze Age to late Iron Age, but apart from this general conclusion the correlation of pollen zones and archaeology in the period from the Early Bronze Age to the Romano-British period awaits a closer analysis.

It will be seen that we have not adopted Erdtman's conclusions with regard to the correlation of the Pennine peats at Warcock Hill, Marsden. This author said that the pollen analyses showed the pigmy flints from the underlying sand to be pre-Atlantic, but we take a different reading of the data. Flints found by Buckley actually *in* the basal peat were of Early Bronze Age type: an actual fragment of

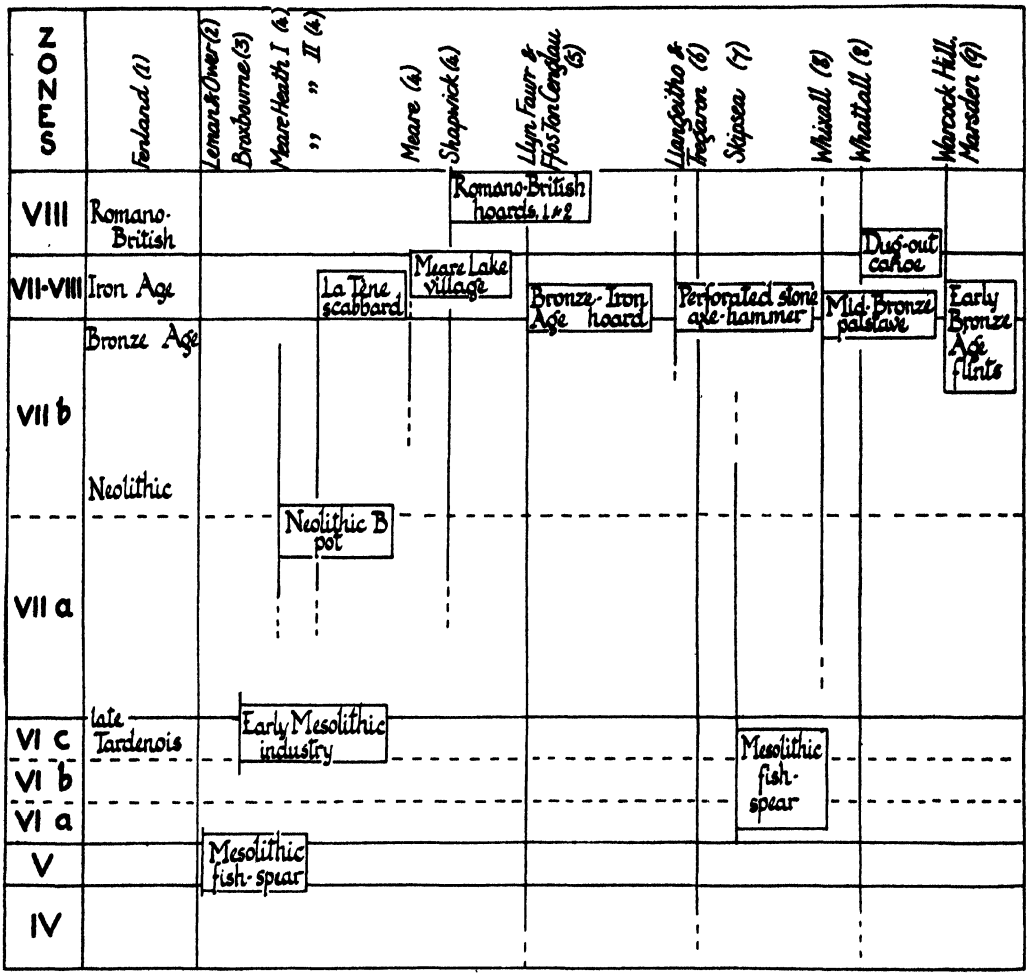


Fig. 13. Table of correlation between forest zones and archaeological horizons.

bronze was recorded there, and Raistrick has since reported bronze celts from similar levels in the district. The pollen zoning is not very evident but suggests the transition at the end of zone VII, and we prefer to regard the evidence as showing that the end of this zone is correlated with the Early Bronze Age: and not in any way related (save by long post-dating) to the pigmy flints, which might very well have been deposited in the sandy soil long before peat formation covered them (Godwin, 1934 & Clark, 1934).

GEOLOGICAL AND CLIMATIC CORRELATIONS

(1) *The late-glacial*

In Ireland Jessen, Mitchell and Farrington have made much progress in investigation of late-glacial conditions and have found good evidence at several sites of the three zones already described by Jessen from Denmark as (i) the Lower Dryas clays, (ii) the Allerød deposits, and (iii) the Upper Dryas clays. The middle of these zones represents a warm stage between two periods in which lake basins were invaded by the typical unsorted material brought down neighbouring slopes by solifluction. There is evidence in the plant content that such layers were formed in arctic conditions, but during the warmer middle period lake mud (nekron mud) accumulated more or less free from inorganic matter and this generally contains seeds and wood of plants which bear witness to the improved conditions. It is interesting to note that remains of the giant Irish elk (*Cervus Megaceros*) were clearly shown to be associated with the Allerød deposits of zone II.

At only one site in England and Wales has a corresponding series been recognized with certainty: this is at Hawke's Tor on the western side of Bodmin Moor, Cornwall, although the results are still unpublished. There are indications however that sites in Shropshire (Hardy, 1939) and Norfolk may yield similar results. In Shropshire and the Lake District in particular it is almost certain that the deposits of innumerable large or small lakes formed behind bars of glacial material contain the accessible history of this most interesting period. The possibility of linking the Irish and Danish results should add further stimulus to our investigation of them. From the bed of Windermere Dr Mortimer has recovered varved clays which it may prove possible to relate to late-glacial series, and we may perhaps expect similar relationships from the Northumberland site where remains of *Megaceros* have recently been found in peat.

(2) *Bog stratigraphy*

During the course of its development a lake becomes filled with inorganic or organic materials and is consistently subject to invasion by vegetation, which alters with changing level of the soil surface in relation to the water-level of the lake. In suitable regions the lake is filled with lake muds, sedge and *Phragmites* peat, and wood peat, and may finally be converted into a large raised *Sphagnum* bog. Throughout this time it is so dependent upon the water level that it is always sensitive to climatic changes of changing precipitation and evaporation. Thus during dry periods invasion of the lake will be accelerated and during a wet period it will be stopped or even reversed. Such changes leave their record in the growing bog deposits. The same sensitiveness is shown by peat deposits not necessarily associated with lakes, such as fens, blanket-bog and the upper ombrogenous parts of raised bogs.

It has been a deficiency in British pollen analysis hitherto that the history of development of the mire should not have been worked out by a close network of borings before samples for pollen analysis have been taken. A few major features have, nevertheless, emerged. One of the most striking of these is the very widespread separation of the *Sphagnum* peat of our raised bogs into distinct upper and

lower layers. The upper "young" *Sphagnum* peat is fresh, unhumified, and pale in colour, whilst the lower is dark chocolate brown, dense, and so strongly humified that little trace of organic structure can be seen in it. A sharp boundary line separates these two layers and peat cutters in different parts of the country all recognize the great difference between the upper, "white", peat which is useful only for litter, and the lower, "black", burning peat. A similar division of peat layers was described by Weber from north-west Germany many years ago, and the term "Grenzhorizont" was given to the sharp boundary between the two layers, where a layer of pine stubs or dense *Calluna* stems often marked the sudden transition. Although it has been said that this phenomenon is not clearly recognizable in British bogs, it is the author's experience (in common with Mitchell and others) that in raised-bogs it is to be found quite commonly, and it is a pity that no convincing English term has been found for this "boundary layer".

The interpretation of the two-fold division is still in dispute. It has been suggested that the "Grenzhorizont" is the expression of the dry Sub-Boreal period, and that the humification of the peat below it is a secondary effect produced during that time. The absence of gradually increasing humification below the boundary layer contradicts the idea of secondary alteration, and we think it much more likely that the quality of the lower peat is due to the primary conditions of its formation and the character of the plant communities which gave rise to it. Despite preconceived ideas as to the climate of the Atlantic period in which much of this lower *Sphagnum* peat formed, it seems to us probable that the raised-bog surfaces were much drier than in the Sub-Atlantic period. The result of recent studies in the ecology of the communities of the raised bogs at Tregaron (Godwin & Conway, 1939) lends support to the view that the bog surface may be very sensitive to climatic change.

There is very general agreement that conditions above the boundary layer were suddenly very much wetter and perhaps colder, and this has been traditionally and no doubt rightly regarded as evidence of a widespread climatic "deterioration" throughout north-western Europe. The period of this climatic change is generally agreed to fall about the transition of the Bronze Age to the Iron Age *circa* 500 B.C., and it corresponds with the end of zone VII in our pollen diagrams, and with the expression of the phenomena of revertence.

There is no reason to doubt that in many British bogs a similar date is valid for the boundary layer, but in others a single clear boundary is lacking, and there are present several surfaces which have this same general character. This condition has already been recognized in southern Sweden by Granlund, who used the term "recurrence surface" (Rekurrenzfläche) for them. He found five of these horizons consistently present in southern Sweden, and was able to date them fairly closely: the middle and most pronounced of them, R.Y. III, he thought equivalent to Weber's "Grenzhorizont". We have not yet in this country been able to recognize Granlund's five recurrence surfaces, but it is interesting to note that Hardy has described three such horizons in the Shropshire mosses, and Godwin and Mitchell have described a "retardation layer" of rather similar character above the boundary layer in the bogs at Tregaron. There is no doubt that the recognition of climatic

changes by this means, the determination of their extent and their correlation with other features of post-glacial history, will be a very profitable future field of investigation.

Closely allied to this stratigraphic evidence of climatic change is the occurrence of layers of tree stumps in bog-deposits. They represent phases of increased dryness wherever they occur over peat, and when they occur on mineral ground below peat they show the effects of increasing wetness. Such changes are usually, but not always, climatic, and they must naturally be brought into relation with other features of bog stratigraphy and forest history. Of particular interest are the forest layers so abundantly associated all over the country with cultural remains of the Bronze Age. Such occur in raised bogs (e.g. bronze palstave in pine stub layer at Whixall, Shropshire), in fens (e.g., pine-yew woods at Wood Fen, near Ely, Cambs) and at the base of blanket bog (e.g. birch woods in South Pennines, Marsden, Yorks). They are exceptionally widespread, and Jessen (1934) has drawn attention to their occurrence very widely indeed under the blanket bogs of Western Ireland, and high on the Wicklow and other mountains also below blanket bog. These pine layers he has shown to have been embedded in peat during the late Bronze Age, which may be contemporaneous with the early Iron Age here. Not only on account of the high altitudes these forest layers reach, but also because they grow in regions which are now desolate waterlogged peat mires, it is concluded that they grew before the great Sub-Atlantic climatic degeneration and were destroyed by this change.

It is inconceivable that so far-reaching a change in western Ireland could have been without effect on the uplands of the rest of the British Isles, and we think that it will be shown increasingly as time goes on that the blanket peat of the Pennines, the Welsh mountains, Dartmoor and Bodmin Moor was very largely the result of the Sub-Atlantic climate, whilst the tree layers so constantly at the base of this peat are somewhat older, in what would be called the Sub-Boreal of the Blytt and Sernander terminology. This is the reinterpretation of Lewis's "Upper Forestian" of the Scottish peat bogs, which was suggested by Samuelsson (1910) and accepted by Erdtman (1928). Unfortunately, pollen zonation of the English and Welsh blanket peats is not yet far advanced: such analyses as exist suffer from lack of detailed long sequences nearby for comparison, but they contain nothing against the view that the greater part of the blanket peat lies in zone VIII. Analyses of this kind include Okement Hill, Dartmoor; Marsden, Yorks (Godwin, 1940, unpublished); Warcock Hill, Marsden, Yorks (Woodhead & Erdtman, 1926); Truckle Pits, Barden Fell, Yorks (Raistrick, 1933); Kilhope Moor, Northumberland, Mickle Fell, Yorks (Raistrick & Blackburn, 1932*a*); Stake Pass (Raistrick & Blackburn, 1932*b*); Heathery Burn Moor, Northumberland (Raistrick & Blackburn, 1931).

The abundance of prehistoric remains high on the plateaux which now carry blanket bog is most striking, particularly with respect to the Neolithic and Bronze Age cultures: extensive peat bog formation must have post-dated this period of prehistoric occupation.

Two further points of interest arise in relation to these forest layers. The first is the prevalence in them of large stumps of *Taxus*, which must have flourished widely

even upon peat surfaces. The second is the failure of this extensive development of pine and birch woods to make impress on the general drift of pollen diagrams of peat bogs, save where such diagrams come from sites actually bearing the forest layer. The pine and birch, it seems, cannot at this time have displaced the native alder mixed-oak forest at all, and it may be supposed that their extension followed invasion of regions and soils previously not available to trees. They thus colonized the margins of peat mires of all kinds as these became dry enough for tree establishment, and extended far up the mountain sides, possibly above the previous tree limit, but in each instance into a region fairly free from tree competition. Both pine and birch are rapidly spreading and tolerant species capable of filling this role.

Such forest layers are thus linked with the "Grenzhorizont" as expression of the dry period just before the Sub-Atlantic climatic deterioration. What remains impossible is any indication of the *onset* of such a dry period, or indeed of the recognition of any period which can be clearly said to correspond with the Sub-Boreal of Blytt and Sernander. Though indications of dryness exist then, as we have already seen, more recurrence surfaces than one may be found and we are inclined to think of all the lower *Sphagnum* peat as indicative of dryness. The question of the Sub-Boreal, in Britain, and the climatic changes in the long period between zones VI and VIII, is one for which present evidence is quite inadequate, but which evidently demands investigation.

(3) *Land and sea-level changes*

The peat beds which occur on our shores, and which yield "moorlog" to the trawls of the North Sea fishermen, are such clear indices of marine transgression that the greatest interest has always been given to them, and legends of lands lost to the advancing sea are common on our western shores. Hitherto very sparse finds of artifacts have been almost the sole evidence on which these submerged beds might be dated, but the development of the pollen-analysis technique at once suggests a tool of great value in their examination. It is only necessary to recall that many of them are "forest beds" in which occur the stumps of trees, to indicate the care which should be taken to allow for local factors in interpreting the pollen analyses from them.

The method has already gone some way towards showing the date of formation of the North Sea. Peat detached from the floor at various known depths and in known places has been analysed by Erdtman, Polak and the author, and the results agree in showing that samples from depths between 100 and 170 ft. below sea-level contain little besides pine and birch pollen and a little hazel. They cannot therefore be more recent than zone V. On the other hand, samples from much shallower water on the North Sea coast contain abundant alder, lime and mixed-oak forest trees and therefore belong to zone VII. The deepest samples, from St German's near King's Lynn, are of this kind and lie at $-23\cdot5$ ft. O.D. Thus the greatest part of the North Sea transgression probably took place between zones V and VII. A closer dating would be possible if dock excavations or borings should at some time yield

for analysis peat samples from a deep channel containing deposits of intermediate depth formed at stages of the transgression itself.

Samples recently analysed from borings in Swansea Bay, South Wales, appear to fulfil this condition (Godwin, 1940*b*). Peat beds or peaty layers there occur at various depths to as low as −54 ft. below sea-level, separated from one another by silts of semi-marine origin. Prof. O. T. Jones has shown that these deposits all lie within the ancient channel of the River Tawe, which before they formed was cutting to a base-level of at least 200 ft. below present sea-level. Pollen analyses of the peat layers has shown remarkably conclusively that those at −54 to −28 ft. O.D. belong to zone VI*b*, and those at −20 ft. O.D. to zone VI*c*. Thus very rapid transgression must have been in progress during at least the second half of zone VI; the rate being of the order of 5 ft. a century. Peat beds exposed at about −5 ft. O.D. on the coast are referable to zone VII, and only a few feet higher are coastal peats belonging to zone VIII. It is therefore evident that the pace of marine transgression slackened at the end of zone VI*c*, and may indeed have been interrupted by marine *retro*gression. Large transgressions at the same time in different parts of the country, such as seem indicated by these results from the North Sea and South Wales, indicate that the transgression is a eustatic movement, due to an absolute rise in sea-level at the same time all over the world. When on the other hand unequal loading of the earth's crust has caused warping, there will be isostatic movement, and it ought to be possible to demonstrate this through pollen-analysis methods, by showing that transgression must have been in progress at a given time at one coastal site, whilst retrogression or a different rate of transgression was in progress at another. In order to establish this it is necessary to be sure of the stratigraphy of sensitive coastal tracts such as deep estuaries abutting on fens or bogs, where the interplay of marine and fresh-water deposition has had full expression and has left the record of former land and sea-level relations. The Fenland of East Anglia has proved fairly suitable to such examination and it has proved feasible to construct a diagram relating former land and sea-level with fair sensitiveness to the pollen zonation. Similar curves for estuarine regions in places such as Southampton Harbour, the Somerset Levels, Swansea Bay, the Dovey Estuary, Morecombe Bay, and the Scottish Carselands would give the evidence needed for resolving former land- and sea-level movement into isostatic and eustatic components. Hitherto observations in such areas, although quite useful, have not been sufficiently detailed for this purpose. When accomplished for this country it would be relatively simple to extend our comparisons to the Netherlands, north-west Germany and the Scandinavian coast, where similar material is being investigated. Post-glacial raised beaches can hardly be related to polleniferous deposits in England and Wales, although the relation has already been partly investigated in Scotland.

(4) *Changes in fauna and flora*

There has not yet been any very systematic attempt to relate our pollen zonations of the late-glacial and post-glacial to the many changes of fauna and flora which have taken place through this long period, but it must be evident from what has been

written above that there are considerable possibilities in it of linking stages of forest development with the knowledge already possessed by taxonomic experts of the former range of animals and plants in many specialized groups. The Fenland studies have been linked with studies of Foraminifera by Dr Macfadyen, of Mollusca by Dr Kennard, of Vertebrata by Dr Jackson, and of Diatomaceae by Chr. Brockmann. Sometimes such studies indicate the prevalent local conditions, such as salinity in the case of the foraminifera. In other instances, as in the fauna found by Praeger (1892) in the Belfast Lough investigations, the subfossil fauna may have a general character indicative of temperature conditions different from those of today.

The dating of the Irish Elk has been already mentioned, but the finds of beaver, wolf, pelican, *Bos primigenius* and other extinct vertebrates has hitherto been more closely related to archaeological cultures than to pollen investigations.

Plant remains, particularly seeds and fruits of aquatic species, are very commonly preserved in lake deposits and have already been mentioned as supplying much evidence for the character of pollen zones I, II and III in the Irish investigations. Such identifications are clearly the most direct means of ascertaining the former range of native species, and no doubt we shall find the ranges of many have altered greatly. Discovery of *Naias flexilis* in the lake mud below Tregaron Bog, corresponding to zones IV or V, illustrates the possibilities. It is also worth recording that Miss Hardy recorded abundant rhizomes of *Scheuchzeria palustris* in peat just above the "Grenzhorizont". Although very abundant then, the plant is now of course very rare in England and Wales, and it is interesting to find an exactly similar situation has been shown to exist in Denmark where the plant is abundant in the early Sub-Atlantic layers of peat bogs but virtually absent as a living species.

We might in conclusion mention the fact which has been pointed out by Blackburn (1938) and by Godwin & Conway (1939) that the bulk of Sub-Atlantic peat in English and Welsh bogs is made of *Sphagnum imbricatum*. As this readily recognizable Atlantic species is now almost absent from the living bog surfaces, it is probable that there has been a recent change in the direction of increased dryness or continentality. The same phenomenon has also been described for the raised bogs of Holland and north-west Germany. Such a climatic change must fall very near the end of zone VIII, and within historic time.

SUMMARY

From a review of the evidence available from sites distributed over England and Wales, it appears that a pollen zonation established in the East Anglian Fenland is applicable to the whole country. This reveals a strong regional differentiation which corresponds with present-day differences: in each part of England and Wales the forest history has followed an equivalent but not identical course. This regional parallelism strengthens the evidence for a phase of revertence in zone VIII, the Sub-Atlantic, in which a returning importance of *Betula* is very widespread.

The successive zones and subzones have been distinguished as follows:

VIII. Alder-oak-elm-birch-(beech) zone.
VII (*a* and *b*). Alder-oak-elm-lime zone.
VI (*a*, *b* and *c*). Pine-hazel zone.
V. Pine zone.
IV. Birch-pine zone.

It has been further shown that the correlation of these zones with archaeological horizons in the Fenland can be consistently extended to the rest of England and Wales, although everywhere the Bronze Age and Iron Age correlations call for more detailed analysis.

The correlations with the stratigraphic indices of peat deposits and lakes indicate the possibilities rather than the achievements of the method, but it is suggested that the blanket bog of our western and northern uplands is chiefly Sub-Atlantic in age. It is also suggested that the "Grenzhorizont" or "boundary layer" is a useful and generally recognizable phenomenon in raised-bog stratigraphy, although the climatic conditions of formation of the lower peat are uncertain and although other "recurrence surfaces" or "retardation layers" exist. The evidence for a specific climatic differentiation of the Sub-Boreal from the Atlantic in this country seems to be slight.

It seems certain that pollen analysis will be of particular value in investigations of submerged peat beds, and there is already strong indication of the rapid marine transgression which formed the North Sea having been in progress during zone VI, the Boreal period.

There is an interesting field of enquiry in relating the former extension of our fauna and flora to the forest history.

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CONTRIBUTIONS TO THE ECOLOGY OF BRACKEN (*PTERIDIUM AQUILINUM*). I. THE RHIZOME

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(With 10 figures in the text)

CONTENTS

Site of observations and experiments

It is possible that the behaviour of bracken in Breckland does not differ fundamentally from its behaviour elsewhere, but the marginal conditions of Breckland may throw into relief phenomena which may be obscured under conditions more congenial to this plant. Certainly its behaviour on Lakenheath Warren at the place marked *Y* in Fig. 9 of Watt, 1937 is arresting: and this arises more from the concentration of several phenomena in one small area than from an obviousness in any one which by itself and diffused over a larger area would tend to pass unnoticed. The phenomena observed here are widespread in Breckland and some of them have been seen elsewhere; for example, ring formation has been seen in Sutherland, in Cumberland (reported to me by Dr M. R. Brown), in Dumfries (communicated in a letter from Mr T. G. G. Powell to Prof. F. T. Brooks), and in the west of Scotland by Braid (1937). This investigation has yielded results which hold good for this area on Lakenheath Warren: how far they are of general application further work must decide.

Brief description of the phenomena: areas D and E

The area (Fig. 1) lies at an altitude of just over 50 ft. O.D. (15 m.) and slopes gently to the north (1 in 100). The bracken is moving north-eastwards into grasslands of types D and E (Watt, 1940): part of type D is dominated by *Carex arenaria*,

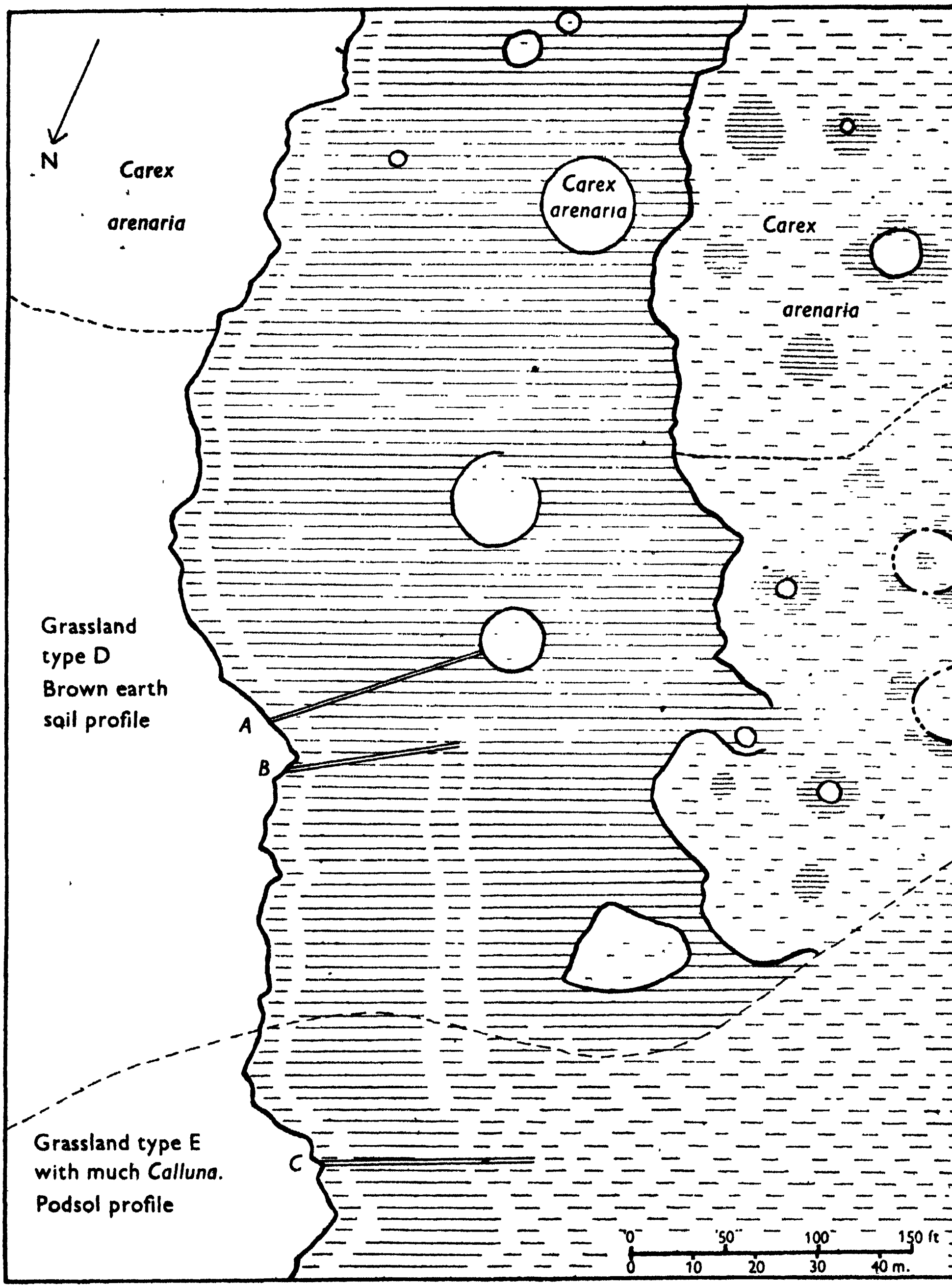


Fig. 1. The Chart shows a part of the area described. Bracken is invading two kinds of grassland provisionally labelled D and E. The long horizontal lines represent areas where bracken is completely dominant: the short where it is not. Between areas D and E there is a transitional zone and the dividing line marks the limit of typical E. The hinterland of area D is represented diagrammatically. A, B and C are transects to which reference is made in the text (vide also Fig. 2).

and the latter is really a patchwork of locally dominant *Calluna* set in a background of grassland E. The behaviour of the bracken differs in the two types and is doubtless related to the soils which characterize them (see description and Fig. 1 in Watt, 1940). Briefly, the soil of grassland D is a brown earth overlying chalky boulder clay at about 3 ft. The soil of type E is a podsol. For convenience these two areas are called area D and area E respectively.

Area D (Figs. 1, 2). The vanguard of the bracken consists of scattered fronds, short, deep set in the soil with short petioles showing above ground. The fronds then increase in number, grow taller, have longer petioles, and eventually form a continuous canopy of live fronds and a mat of dead fronds covering the ground and killing the grass. The zone up to the point where continuous canopy is formed is referred to as the advancing margin. Behind, the fronds become still more numerous and taller with longer petioles: in fact, the fronds reach their maximum height here forming the crest of a wave advancing on the grass heath. Thereafter the fronds are somewhat shorter but are still tall with long petioles, and a relatively uniform height and density are maintained over a considerable but varying width. From the point where canopy is formed to the further end of this zone of uniform height the bracken is completely dominant. Then the dense and tall bracken ceases abruptly along a wavy line giving way to a hinterland in which the bracken is patchy: there are patches where the fronds are few and short with short petioles, others with more and taller fronds with longer petioles, and still others with fronds reaching the average height of the dense uniform belt. In some of the patches these three phases grade into each other forming roughly concentric zones round a nucleus of tall bracken. In the centre of some of these there are small circular areas free from fronds, in others larger circular areas with few scattered short fronds, and there is the same abrupt change in height and number as in the change from the belt of uniform bracken to the hinterland of patchy bracken. In fact, the phenomena of the patches in the hinterland largely repeat those of the main body with this difference, that the various zones instead of being in alignment with each other are concentric.

Set in the uniform bracken of the main body there is a number of rings of varying size. In 1932, some of these at least, if not all, had no fronds. Subsequently (in 1935?) many fronds appeared, but again there is the same sharp break from the tall dense bracken of the main body to the short scattered fronds of the ring.

Towards area E some of the phenomena become less sharply defined. Transect B (Figs. 1, 2) runs from the margin to a narrow belt of shorter and less continuous bracken, but beyond the end of it the bracken again becomes taller and is characterized by continuous canopy and uniform height.

It should be noted that the grass heath and the soil of the hinterland in which the patchy bracken is set is the same as that beyond the front margin of bracken advance.

Area E (Figs. 1, 2). As in area D, there is an advancing margin succeeded by a broad crest with the same kind of change in number of fronds, height and length of petiole. But beyond the crest the height of the fronds falls to a low, apparently uniform, level in the hinterland. There is no broad zone of uniformly tall bracken

behind the crest. There is no abrupt change in height along any line parallel with the advancing edge, nor are there any large rings. In the zone of maximum height and for some 20 ft. (6 m.) beyond, the canopy and the mat of bracken litter are continuous: thereafter both canopy and mat are discontinuous: there is patchiness but the pattern is much finer than in the hinterland of D.

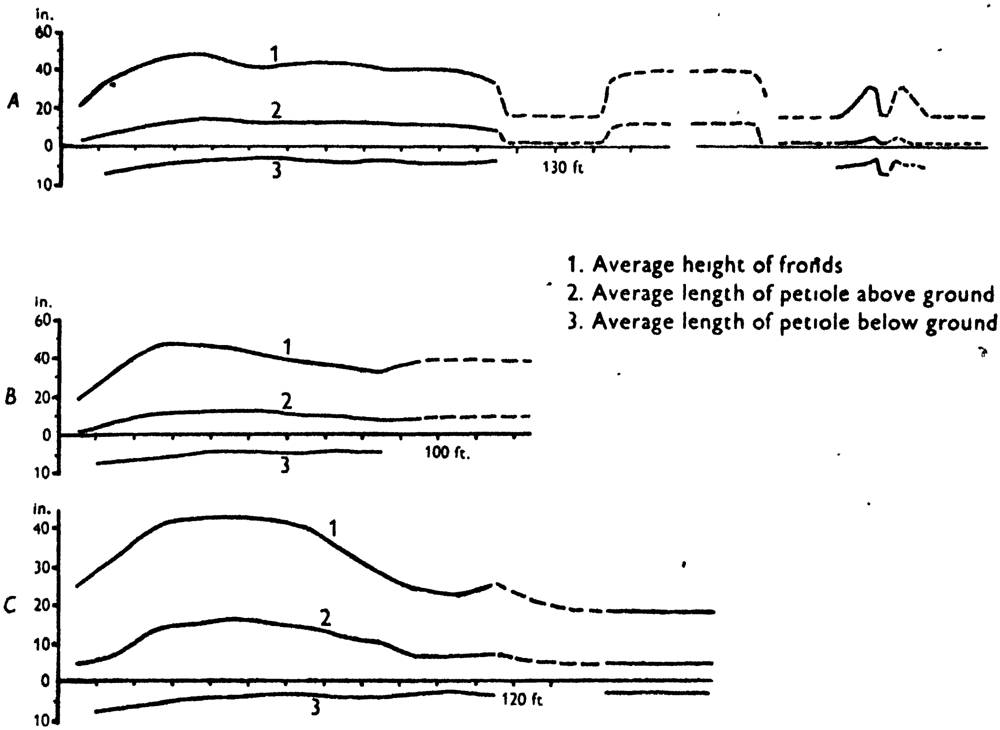


Fig. 2. Transects A, B and C showing the height of the fronds, the length of the petiole above ground (based on averages of data for 1936–9) and the length of the petiole below ground (data for A obtained in 1935, for B and C, 1934). The data for the ring in the hinterland of D (see Fig. 1) were obtained in 1936. The continuous lines are based on the data, the broken lines on estimates.

The plants of the grass heath are entirely killed by the belt of dominant bracken but they reassert themselves in the hinterland, forming a vegetational facies in which the species are the same although their relative abundance is altered by the partial and varying shade cast by the bracken. *Calluna* exists in small and few patches only.

THE CHEMICAL APPROACH TO THE PROBLEM

The rise and fall in the height of the bracken suggest the operation of some factor present in limited amount. That deficiency in the common bases or a fundamental change in the soil is an unlikely explanation is shown by the data in Table 1, obtained from surface (0–3 in., 0–7·6 cm.) composite samples. Nothing in these data points to a change in the chemical condition of the soil to account for the observed changes in the behaviour of the bracken. Nor did any significant differences show in a series of plots in hinterland E manured with different combinations

of fertilizers containing nitrogen, potash and phosphorus. It therefore seems unlikely that an explanation of the phenomena is to be found along these lines, although the possibility exists that some of the rarer essential elements may be limiting. The fact that bracken continues to grow, although reduced in number and vigour, also points to some alternative explanation.

Attention is now directed to the bracken itself to see whether its behaviour can be clarified by an investigation of its morphology.

Table 1. *Some chemical data from surface (0–7·6 cm.) composite samples in different zones of bracken in areas D and E*

	Area D				Area E		
	In grass-land D	In zone of maximum height	In dense uniform bracken	In hin-terland	In grass-land E	In zone of maximum height	In hin-terland
*p*H (Kuhn)	4·2	4·0	3·8	4·0	3·6	3·8	3·7
Carbon %	1·785	1·425	1·740	1·610	2·205	2·190	2·250
C/N	19·40	19·79	19·12	19·63	23·96	23·80	24·19
Exchangeable Ca in M.E.	1·44	1·28	1·28	1·36	1·12	1·12	1·12
Total exchangeable bases	2·40	2·00	1·61	1·61	1·60	1·44	1·44

The morphology of the rhizome

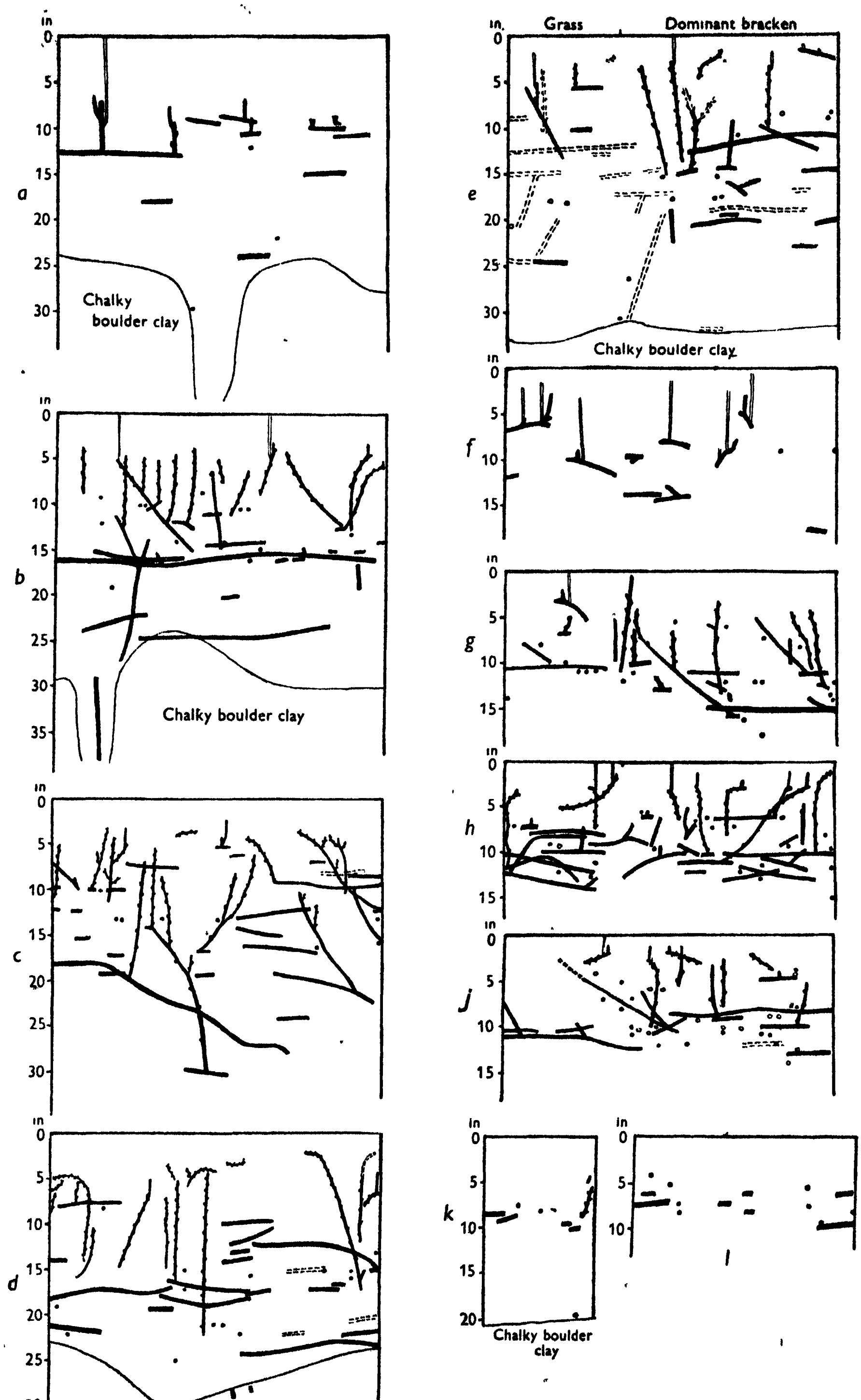
Vertical distribution of the rhizome in the soil

The bracken investigated by Kujala (1926) in Finland had a rhizome which varied in depth from 5 to 15 cm.: but in the figure accompanying his account he shows the main rhizome running at a uniform depth. Smith (1928) gives a maximum depth from Boghall near Edinburgh of 18 in. (45·7 cm.): Woodhead (1906), without giving data, implies that the rhizome runs at different depths in the competitive "association" of the Xeropteridetum and the complementary "association" of the Mesopteridetum, while Braid (1935, 1936, 1937) depicts the rhizome of bracken in the west of Scotland as layered or showing a tendency in that direction with a maximum depth of about 20–25 in. (51–64 cm.).

These and other facts mentioned in the literature need not be questioned: they mean simply that the rhizome is plastic and that its distribution in the soil is a function of the local conditions (climate, soil and vegetation).

This conclusion is confirmed from Breckland. Fig. 3*a*, *b*, *c*, *d*, *e* shows the distribution of the rhizome in area D; *f*, *g*, *h*, *j* in area E; *k* and *l* at Maidscross Hill and Codstone Hill respectively. Other profiles exposed in each of these areas show that the distribution of the rhizome is consistent within each area.

In the pits dug in series across the advancing wave of bracken in areas D and E and in the respective hinterlands, the distribution of the rhizome was charted to a horizontal depth of 3 in. (7·6 cm.). Fig. 3*j* is an exception: the rhizomes (live and dead) in these pits were so numerous that a congested picture would have been obtained: only those exposed on the side to a horizontal depth of an inch or so were charted.



a
b
c
d
e
f
g
h
j
k
l
in
Grass
Dominant bracken
Chalky boulder clay

Fig. 3.

The kinds of distribution already recorded are shown in these figures, and they emphasize the power of accommodation of the bracken and the impossibility of generalizing about the vertical distribution of the rhizome except in terms of the conditions. The interesting problem presented here has not been followed up, but it may be pointed out that in the podsol of E the limit of penetration by the rhizome is generally reached some distance above the B horizon of the soil. The question of a consolidated B horizon acting as a physical barrier therefore does not arise. The barrier is more likely to be physiological than physical, for the rhizome can penetrate fairly compact chalky boulder clay, and on Codstone Hill there is no physical barrier to the rhizome whose maximum observed depth is 10 in. (25 cm.). The approach to an understanding of this varying behaviour is more likely to be through a study of the soil-water regime and aeration as affected by the vegetation itself and by the soil and climate. It may be added as a point of interest that the height of the fronds is not necessarily correlated with the total depth to which the rhizome penetrates.

The length of rhizome per unit area of soil surface

The tangle of rhizome in the soil is revealed by Smith's data (1928) for the total length below a surface area of 2×2 ft. down to the limit of rhizome penetration. In four separate pits he found lengths of 52, 56, 82 and 99 ft. (=396, 426, 625 and 754 cm. per 1 sq. ft. [0·093 sq. m.]). In area E the data from 1 sq. ft. from the advancing margin, the zone of maximum height, in dense bracken at 70 ft. (21 m.) from the margin are 257, 871, and 834 respectively, and in the patchy bracken of the hinterland the average per sq. ft. of fourteen samples each 1 sq. ft. in area is 655 cm. The conditions are probably not strictly comparable, as presumably Smith was dealing with a stable phase like the hinterland of E, but the data happen to be of the same order of magnitude. No data have been obtained from D or elsewhere in Breckland, but Fig. 3 suggests that there must be a very considerable range of variation between the different areas.

In Smith's data no separation is made between the lengths of the main rhizomes and those of the frond-bearing laterals. Table 2 gives those data for area E as well as the average diameters obtained by measuring the diameters of the two ends of each piece of rhizome dug out of the pits.

For the main rhizome the maximum length occurs in the zone of maximum height of fronds: thereafter the data for length falls. The length of the frond-bearing laterals, on the contrary, rises from a small figure in the advancing margin to a

Legend of Fig. 3.

Fig. 3. The vertical distribution of the rhizome in different areas in Breckland. Within each area the type of distribution is consistent but between the types there are obvious differences which relate chiefly to layering and the range of depth covered by the different kinds of rhizome. The solid black represents live rhizomes, the broken lines dead rhizomes and the continuous open (and vertical) lines old or fresh fronds.

a, b, c, d, e are from area D, (*a*) the advancing margin, (*b*) the zone of maximum height, (*c*) at 55 ft., (*d*) at 100 ft. from the front margin and (*e*) the transition from dense bracken to open bracken in a ring; *f, g, h, j* from area E, (*f*) in the advancing margin, (*g*) in the zone of maximum height, (*h*) at 56 ft. from the front margin and behind the zone of maximum height, and (*j*) in the hinterland of E; *k* and *l* are from Maidscross Hill and Codstone Hill respectively.

relatively uniform figure in the zones behind. As to the diameters of the two kinds of rhizomes the data show a marked parallelism, with maxima in the zone of maximum height of fronds: there thus appears to be a general correlation between the height of the fronds and the average diameter of the rhizomes.

Table 2. *Total lengths and average diameters of main and frond-bearing rhizomes from pits, of surface area of 1 sq. ft., in different zones*

Position of pit	Advancing margin	Maximum height	At 70 ft. from front margin	Hinterland	Hinterland (average of 14 sq. ft.)
Main rhizome					
Length in cm.	177	599	491*	218	393
Average diameter in mm.	10·9	13·9	13·0	9·7	—
Frond-bearing laterals:					
Length in cm.	80	272	293*	275	262
Average diameter in mm.	6·8	8·1	7·2	5·7	—

* To the sum of these two must be added 50 cm. length of unclassified rhizome intermediate between the two kinds of rhizome here distinguished.

The size of the bracken plant

If all the rhizomes present in the zone of maximum length survived, then the length of rhizome per unit area ought not to fall as the bracken ages. The fall is due mainly to the death and decay of the main rhizome (vide the open lines in Fig. 3) and suggests that bracken may be a travelling geophyte whose rhizome dies away behind as it advances in front. Bower (1928) and Smith (1928) have stated that this is so without advancing proof. Kujala in Finland and Büsgen (1915) in Germany excavated 4 and 3·85 m. of rhizome respectively without however reaching a decayed end.

Fig. 4*b* shows the length and zigzag course of a main rhizome traced back from the advancing margin to the point where the rhizome decayed (area E). From the apex to the dead end the rhizome measured 67 ft. 4 in. (20·5 m.). Another rhizome traced back in the same way gave a length of 89 ft. (27·1 m.) before the decayed end was reached.

The whole plant in the wave of bracken advance was not exposed and it is impossible to say how large it is. One of the laterals (*X*) was excavated in full. It is shown in Fig. 5*a*. The total length of its rhizome, excluding short laterals but including long laterals, is 200 ft. (61 m.). It does not necessarily follow that all the laterals from the main axis are branches of this size.

In the hinterland the complete plants are smaller: in area E one of 93·1 ft. (28·4 m.) was exposed (Fig. 5*b*) and in area D three complete plants had main rhizome lengths of 2·5, 15·5 and 38 ft. (0·7, 4·7, 11·6 m.) respectively. These last three were excavated from open bracken and are believed to be much below the average size of the bracken plant in the hinterland of D.

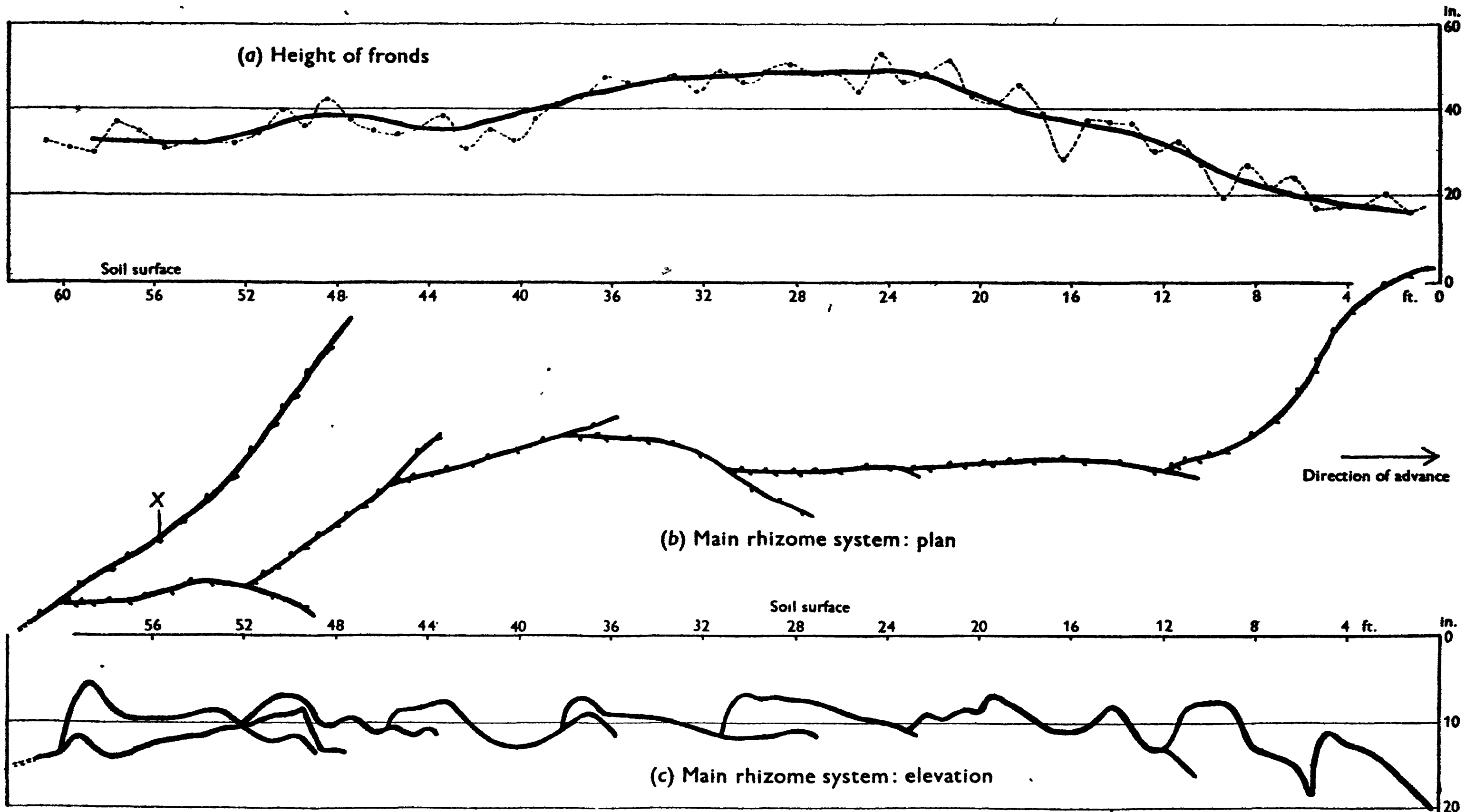


Fig. 4. (*a*) the average height of fronds across the advancing margin of E where the main rhizome was exposed from the invading apex to the decayed end; (*b*) plan and (*c*) elevation of main rhizome. The main axis forms a sympodial system whose segments in general first grow towards the soil surface and then descend. *X* marks the position of the lateral shown in Fig. 5(*a*).

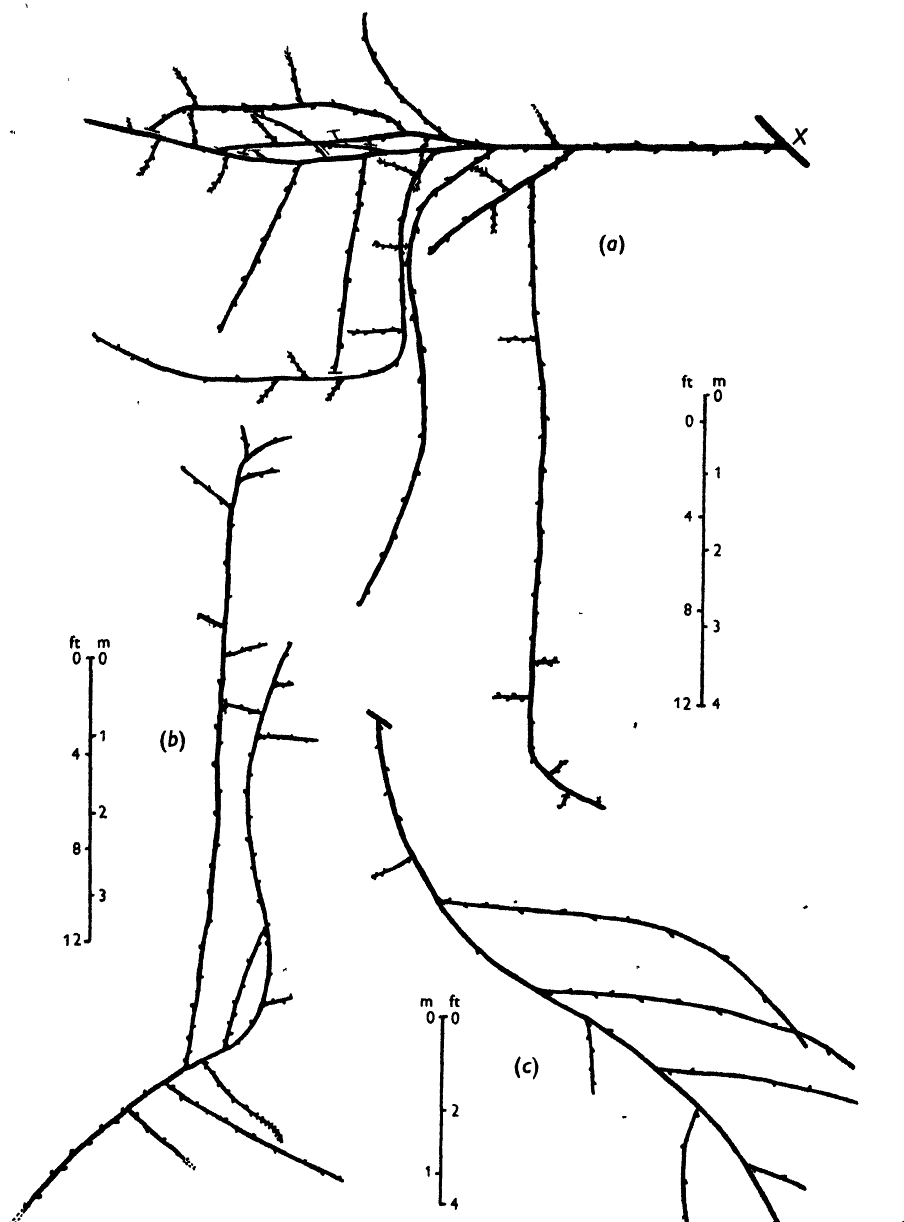


Fig. 5. (*a*) the main rhizome of the lateral at *X* in Fig. 4*b*; (*b*) the main rhizome of a complete plant from the hinterland of E; (*c*) the terminal segment of the invading rhizome in Fig. 4*b* showing the disposition of the long laterals. The short laterals are not drawn, their positions only are marked.

The number of bracken plants per unit area

In an area of bracken there are therefore many plants and not one large one. Examining the available data from the hinterland of E, some very rough idea of the number per acre may be obtained in two ways: (1) by comparing the length of rhizome per unit area with the average length of rhizome of an individual, and (2) by finding out the number of fronds per unit length of rhizome and computing the number of individual plants from the number of fronds per unit area.

(1) By the first method, assuming 50 ft. as the average length of the main rhizome of an individual and 12·9 ft. (393 cm.) the average length of main rhizome per sq. ft., we get a total number of 11,240 plants per acre (=27,770 per hectare).

(2) One of the striking results from the analysis of lateral X (vide p. 421) is the small number of fronds per unit length of main rhizome. Only 12 fronds were recorded, and if allowance is made at the same rate for the severed shoots, the total for the 209 ft. of main rhizome works out at 17. Similar data were obtained from the rhizome excavated in the hinterland of E: 11 fronds per 93·1 ft. A slightly higher number per unit length was borne by the youngest segment growing in the advancing margin, namely, 7 fronds per 41·2 ft. of main rhizome. This gives an average of approximately 1 frond to 10 ft. of main rhizome. Now the average number of fronds per 10 sq. ft. is 21·6 (based on the number of live fronds in August in 12 plots each of 16 sq. ft. over the years 1932–8 inclusive). Again assuming an average individual to have 50 ft. of main rhizome and to bear 5 fronds, we get the astonishing total of 18,810 individuals per acre (=46,500 per hectare).

Although the two results differ, the significant fact remains that there are many thousands of independent plants in an area of the size of an acre, and the number would still be of the order of thousands even if the basic data for size of individual and number of fronds were doubled.

The architecture of the rhizome: long and short shoots

Fundamentally the bracken rhizome forms a sympodial system (Fig. 4*b*, *c*) from the segments of which lateral branches are given off, with rare exceptions, alternately right and left. Typically and for convenience two main types of shoot may be distinguished, long shoots with an average internodal length of about 30–40 cm. and short shoots bearing fronds with internodes of 0·5–2 cm. Between these types there are all gradations of internodal length, and it will be convenient to refer to these as intermediate shoots, but structurally they differ from long shoots only in the length of the internode. An arbitrary upper limit of 15 cm. is used where a distinction is made between intermediate and long shoots. Neither of these types bears fronds: only the short shoots appear to bear fronds directly on the rhizome.

The invading rhizome (Fig. 4*b*) pursues a zigzag course and consists of a series of segments forming the main axis whose continuation is maintained by lateral branches arising behind the apex of the segment and at a varying distance from it. The parts of the segments forming the main axis are remarkably uniform in length, 5 of them approximately 7–8 ft. (2·1–2·4 m.), although the total length of each

segment from its base to the apex varies from about 9 ft. to at least 20 ft. (2·7–6 m.). The total length of these complete segments is 101 ft. (30·8 m.). A sympodial structure is also found in the rhizomes of the advancing margin in D and on Codstone Hill.

In the invading plant (Fig. 4*b*) most of the apices of the segments were thickened and sclerosed and showed evidence of insect damage: one was cut across. Whether the insect damage happened before cessation of elongation and was the cause of it or whether after growth had ceased could not be determined. The point is obviously important because the whole sympodial system presented may have been due to injury to the growing points. The matter was therefore investigated further only to find that the same sympodial system is developed where the apices of the segments are alive and still growing or swollen, sclerosed and intact.

Normally branches are given off right and left alternately to form in new soil a regular pattern in which the branches become older with distance from the apex, but not necessarily longer. In fact, branches from near the base tend to remain either dormant or short, while those from the middle or near the apex become long (vide also Fig. 5*c*), technically either intermediate or long shoots. These in turn give off the frond-bearing short shoots in the same regular sequence.

Where the bracken is not in competition with its fellows the pattern formed is remarkably regular. This of course applies to the system across the advancing wave of bracken where the general course of the main axis is perpendicular to the line of advance. And the whole wave may therefore be visualized as composed of a series of axes running more or less parallel to each other, and each successive zone parallel to the front margin will contain bracken at about the same stage of development. Regularity also characterizes the general plan of the rhizome in Fig. 6*b* from the hinterland of D in a place where it was the sole occupant of the ground. Where, however, there is competition with other bracken plants, there is no regularity either in the direction of growth of the main axis or in the production of short and long laterals. Thus in the youngest segment of the bracken invading grassland E there are 7 long (and intermediate) shoots to 7 short on the 15 ft. of main axis: in the hinterland there are 9 long (and intermediate) to 25 short shoots per 25 ft. of main axis.

Long shoots which are long shoots from the first arise by branching only from long shoots: shoots of intermediate length arise from intermediate and long shoots: short shoots arise from any category including short shoots. But a shoot does not necessarily belong to one category throughout its length, in fact short shoots may grow into long shoots (Fig. 6, and Kujala's figure) and long shoots may end in short shoots (Fig. 5*b*) each with intermediates between the types.

Thus in Fig. 4*b*, where the short lateral projections mark the nodes, the internode in general lengthens with distance from the base of the segment, the average lengths of the first 12 internodes of 7 segments being 2·5, 16·0, 15·3, 21·5, 20·5, 23·5, 24·0, 31·8, 36·0, 32·8, 36·8 and 40·0 cm. Excepting one segment the first internode is short: the next two internodes in most are intermediate, the rest are long. And in general, even where the segment is a long shoot throughout, the first internode is

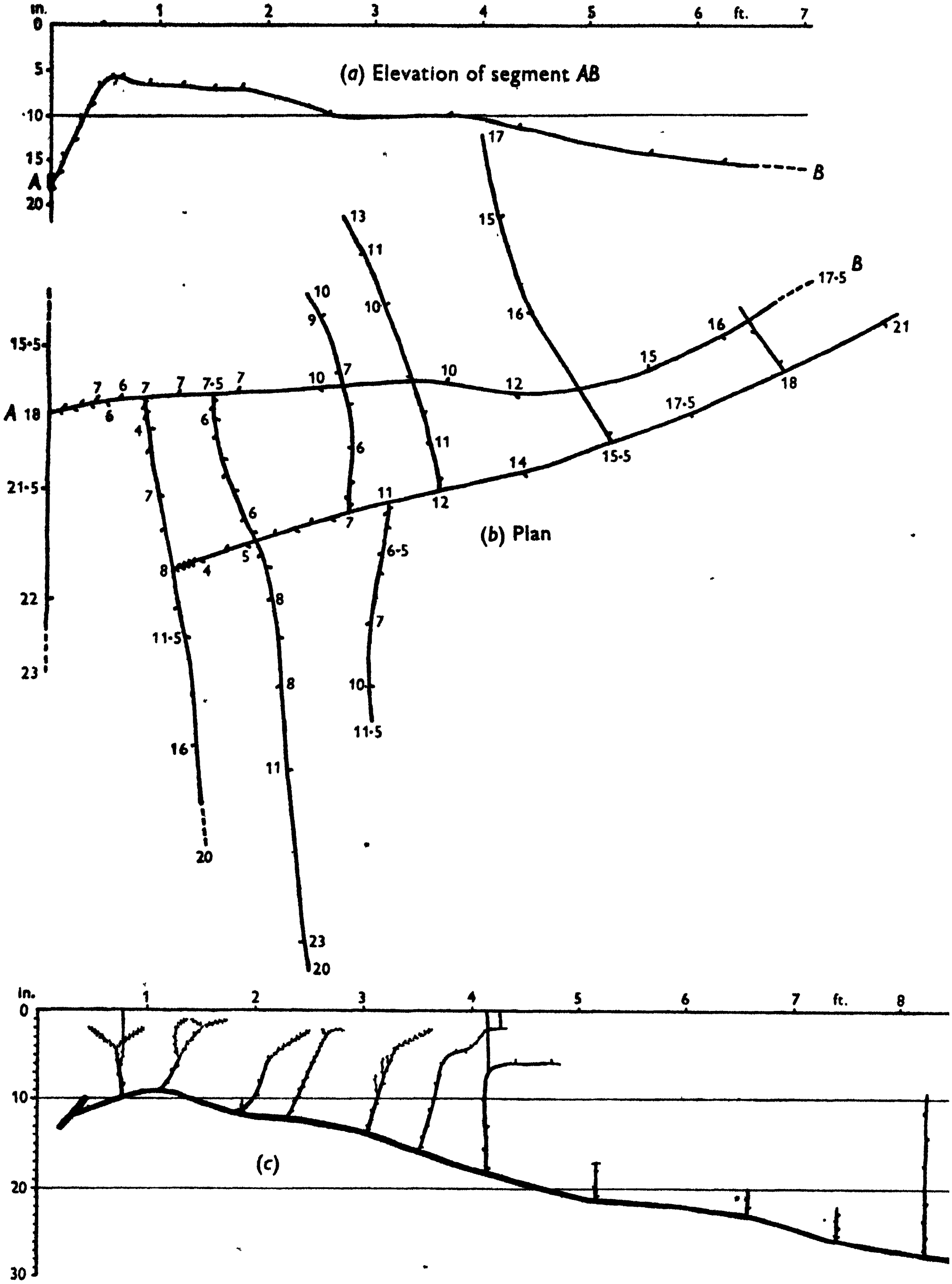


Fig. 6. (*b*) A complete plant from a patch with few fronds in the hinterland of D. Note the extension of short shoots to form long shoots and the correlated change in depth (*a*). The numbers along the rhizome are depths in inches. The symmetrical plan is probably related to freedom from competition with other bracken plants: cf. Fig. 5*b*, where the plant grew in competition with others. (*c*) The elevation of one of the long shoots of the plant shown in Fig. 5*b* giving details of the short shoots.

usually short (Fig. 6*b*), and in the same figure are to be found examples where both short shoots and intermediate shoots have become long shoots. There is no evidence that internodal length normally falls towards the apex of the segment, although of course there are examples where the long shoot becomes an intermediate and even a short shoot. The maximum internodal length measured is 69 cm.

Occasionally a relatively short internode is interposed between two long ones as in the oldest segment at *X* (Fig. 4*b*) and in the middle of the terminal segment. In the latter the rhizome bends sharply upwards at this point (and incidentally two successive nodes are found on the same side) simulating a lateral in behaviour (vide next paragraph but one). No such change in behaviour is found in the oldest segment.

There does not appear to be any relation between the internodal length and soil type: thus in the main axes of rhizomes advancing into grasslands D and E the average length of the terminal 28 internodes of 15 cm. and over is 35·0 and 36·6 cm. respectively: on Codstone Hill where the bracken is short and open the terminal 12 internodes averaged 38·1 cm. On the other hand, 28 internodes in the main axis of the plant examined in hinterland E averaged only 26·9 cm.

In general, the three categories of shoot behave differently. Long shoots run more or less in the same plane as the parent axis and in the deeper soil layers: intermediate shoots grow very obliquely upwards from a parent long shoot, then run horizontally at a higher level: short shoots grow perpendicularly or obliquely upwards until they come to within 2·5–10 cm. of the soil surface, then turn parallel to it. And this change of behaviour marks the change from a long shoot to a short shoot or vice versa: the short shoot turns up from a lower level in the soil, the long shoot down from a higher. This difference in behaviour is seen in Fig. 4*c*, where in general each segment begins by rising towards the soil surface, then descends with increasing length of internode. The same phenomenon is depicted in Fig. 6*a*, *c*.

While it is convenient to recognize this general relationship between the kind of shoot and its average depth, there is of course a good deal of overlapping incidental to the depth of the point of origin of the shoot: short shoots, for example, arise not only from intermediate shoots at an intermediate depth but also from deep long shoots. Nevertheless, the relationship may be detected even where the whole rhizome system is compressed to occupy progressively narrower and shallower zones in the soil. Thus in the deep soil of D the long shoots are found over a considerable range of depth from about 30 cm. to a maximum measured depth of 96·5 cm.: in E this zone is telescoped, occupying a layer from about 23 to 38 cm. from the soil surface. On Codstone Hill the long shoots run at an average depth of 18 cm. (with a range from 13 to 21·5 cm. only in 6 m. of rhizome) just under the intermediates at about 13 cm. In this last area the short shoots form a small angle with the horizontal, that is, they move only slowly out of the plane of their parent rhizome: in D and E the angle is wide.

These facts present problems which are not yet solved. Just what determines whether a bud will grow into a long, intermediate or short shoot is no doubt the resultant of a complex of causes comprising both the internal state of the plant and

the external conditions (including competition with other bracken plants and other types of vegetation) at the time of elongation. Further, the causes of variation in depth of the main axis as well as the movement of the main axis within a certain range of depth in each soil type are unknown. But it appears as if external influences operating at the time of extension were largely responsible, sometimes apparently inducing the rhizome to move into a soil layer suitable for a time only. When, later, the conditions become unsuitable the growth of the rhizome is arrested or the apex decays. Thus in Fig. 6*b* the apical parts of two long shoots had decayed at depths of 43 and 51 cm. respectively. Other apices are however alive and healthy at 51 cm.

The short shoot

The shoots of intermediate length are not dealt with separately. They repeat the morphological features of the long shoots, as in fact do the short shoots except that they grow upwards (in D and E) and appear to bear the fronds directly on them. It is these short shoots which are figured by Sachs (1882) and Smith (1928). Two are figured here (Fig. 7*a*, *b*).

Five shoots with the same number of internodes had the following average internodal lengths from the base to the apex: 2·90, 4·98, 3·30, 2·26, 1·72, 1·50, 1·30, 1·16, 1·26, 1·16, 1·06, 0·98, 0·88, 0·78, 0·82, 0·70, 0·68, 0·64, 0·78, 0·76 and 0·74 cm. The average internodal lengths of 50 shoots of unequal length show the same kind of change: the first internode has an average length of about 2 cm., the second about 4·5 cm. This is the maximum: thereafter the length falls quickly then slowly to a more or less constant value from about 0·56 to 0·70 cm. At the same time the shoots become thinner, in some down to a diameter of 0·30 cm. only at the apex.

As in the long and intermediate shoots, the first bud is always produced on the adaxial side: the rest with rare exceptions are found alternately right and left. Here again the first bud is often dormant, but other and higher buds may remain so. In Fig. 7*a* all the fronds appear to have arisen directly from the rhizome, as do those in the upper middle portion of Fig. 7*b*. But inspection of the lower part of the rhizome in Fig. 7*b* shows a progressive shortening of the lateral frond-bearing axes until it finally disappears altogether. Towards the apex of the same shoot the internodes of the main axis have again become longer and the basal internode of a lateral axis has also become evident. Again, the basal lateral shoots which bear the frond have grown beyond the frond insertion although remaining dormant: higher up they are shorter and smaller until finally they are not produced at all or are represented by a bud which appears to be inserted on the base of the frond.

If we agree with Velenovsky (1905)—and his conclusions in this matter are acceptable to Bower (1928)—that the rhizome is a dichopodium in which one branch is relatively undeveloped compared with the other and thus appears lateral to it, it seems difficult to avoid the conclusion that the short shoot is a weak and telescoped long shoot in which the "laterals" are suppressed. The bud at the base of the frond which has been the subject of discussion ever since Hofmeister's time (1862) represents an undeveloped lateral.

Under normal conditions these buds at the base of the frond usually remain

dormant, but in the upper part of the shoot and hence near the soil surface some give rise to short shoots still more slender than the parent axis but like them grow erect or obliquely upwards. Thus in the hinterland of E, where more buds of this kind

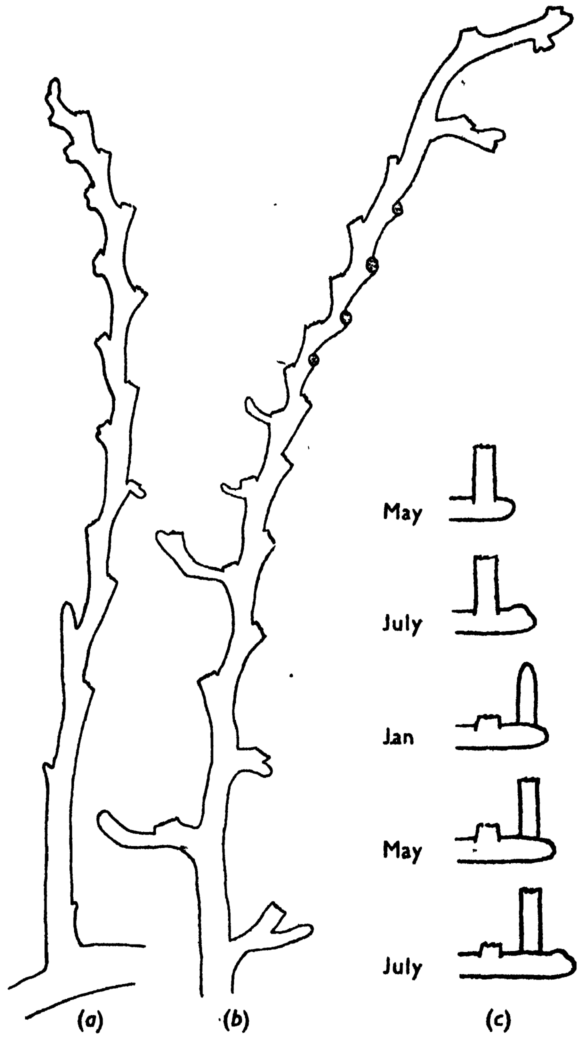


Fig. 7. (*a*) and (*b*) are two short shoots. In the lower part of (*b*) the progressive shortening of lateral axes is shown until the frond appears to arise directly from the main axis and the lateral axis is then probably represented by a bud which in some has developed into a lateral shoot, the frond base then appearing axillary, (*c*) diagram based on the data given on p. 420 showing the extension of the short shoot and the growth of the young frond over a period of one year. The frond grows to maturity about 12 months after its first visible appearance.

develop than in D, the short shoots usually remain unbranched at the base but some branch fairly freely towards the apex, the branches growing erect or spreading more or less fanwise in a horizontal plane below the soil surface.

In this area (D and E) the short shoots grow upwards, then bend and grow parallel to the surface for 5–10 cm. at depths varying from 2·5 to 10 cm. In quite a number the course upwards is not uniform, and after growing horizontally for some distance they again turn upwards to grow horizontally once more on near approach to the soil surface. Incidentally most of the roots of the upper parts of short shoots grow towards the surface of the soil: on the parts of shoots which are horizontal the phenomenon is less marked, more roots growing in directions other than upwards.

The fate of the short shoot and the origin of independent plants

As the main rhizome dies away behind, the laterals will be thrown upon their own resources. Smith (1928) has in fact suggested the origin of independent plants from short laterals in this way, but in this area the usual fate of the short shoot is death from the apex downwards, while the part of the parent axis to which it is attached is still alive. The rate of dying back appears to be slow, and the basal part of a short shoot may sprout from dormant buds while the apical part is dead (Fig. 6*c*). On the parent rhizome the older short shoots usually die first, but this is not absolute and dead dwarf shoots may be flanked by live ones. On the other hand, a few of the short shoots may grow into long shoots, the rhizome descending obliquely in the soil. It would thus appear that it is the long shoots which by decay of the parent axis give rise to the independent plant.

The growth of the invading long shoot

In west Scotland the long shoot extends from 1 to 3 ft. (0·3–0·9 m.) per annum (Braid, 1936, 1937): in Finland 20–30 cm. (Kujala, 1926). Marked shoots invading grasslands D and E gave the following data: in D, 1936–7, 112·0, 79·9 and 96·7 cm., the first two of these grew 58·3 and 90·3 cm. respectively in the following year: in 1939–40 (a year when fronds were severely crippled by spring frosts) lengths of 63·5 and 47·0 cm. were measured. In E, only two measurements are available, 40·6 cm. in 1936–7 and 25·4 cm. in 1939–40. The rate of extension is thus appreciably less in E than in D, in fact an average of 33 cm. compared with 74 cm. per annum. No measurements are available from the hinterlands of D and E, but under competitive conditions the rate is probably much less (*vide*, p. 422).

The development of the laterals varies from shoot to shoot: in some, one undifferentiated lateral only may appear, in others laterals may be so far developed as to carry well-developed fronds (*c.* 5 cm. long) in March—fronds which but for the severe frost in early 1940 would have emerged in the spring (Fig. 8). Thus the differentiation of the frond and its visible appearance have taken place within the year. The apex of the invading rhizome is of course some distance in advance of the first frond to emerge above the soil surface: variations from 22 in. (56 cm.) to 51 in. (129·5 cm.) have been noted.

Rate of growth throughout the year

Three shoots in the advancing margin of D were measured periodically from 14 May 1936 to 14 May 1937. The averaged results are plotted against time in Fig. 9, and weekly rates are tabulated (Table 3). Growth did not cease during any

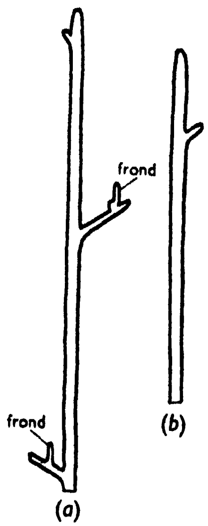


Fig. 8.

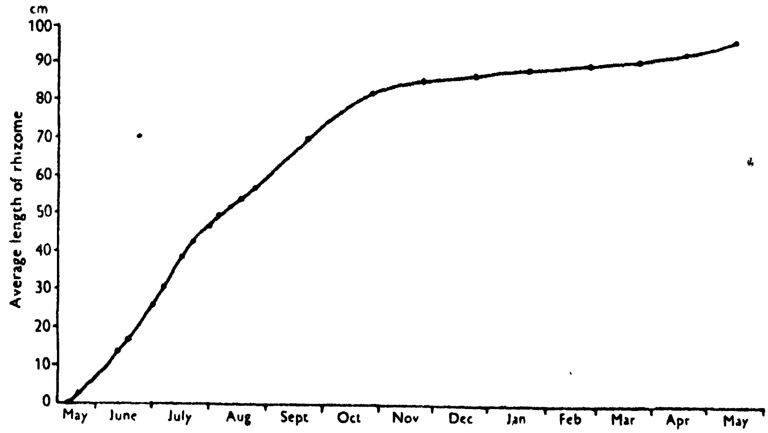


Fig. 9.

Fig. 8. Extension growth during the year March 1939 to March 1940 of two rhizomes invading grassland D showing different degrees of development of the lateral shoots: in (*a*) two fronds are well developed (March 1940).

Fig. 9. The average extension rate of three rhizomes invading grassland D during the year May 1936 to May 1937.

Table 3. *Growth in length of rhizomes invading grassland D during the year 1936–7. Weekly rate of growth and temperature summation over 36° F.*

Period	Number of days in period	Extension in cm. during the period	Weekly rate of growth in cm.	Summation of ° F. over 36° F. during the period (air temperature at Elveden Hall)
1936: 14. v–21. v	7	2·71	2·71	153
21. v–11. vi	21	10·58	3·53	322
11. vi–18. vi	7	3·36	3·36	161·5
18. vi–30. vi	12	9·32	5·44	365
30. vi–6. vii	6	4·24	4·95	161
6. vii–16. vii	10	8·33	5·83	256
16. vii–22. vii	6	4·12	4·81	151
22. vii–30. vii	8	3·84	3·36	183·5
30. vii–4. viii	5	3·00	4·20	122·5
4. viii–12. viii	8	2·37	2·07	182
12. viii–17. viii	5	1·70	2·38	132·5
17. viii–25. viii	8	3·43	3·00	224
25. viii–22. ix	28	13·20	3 30	873·5
22. ix–26. x	34	11·93	2·46	460
26. x–24. xi	29	3·50	0·85	237
24. xi–22. xii	28	1·17	0·29	130
1937: 22. xii–21. i	30	1·83	0·43	139
21. i–22. ii	32	1·17	0·26	194
22. ii–22. iii	28	0·97	0·24	99
22. iii–16. iv	25	1·53	0·43	209
16. iv–14. v	28	3·90	0·98	387·5

month of the year, but two phases are well marked, a period of fast growth from the middle of May to near the end of October and one of slow growth during the rest of the year.

Rate of growth and temperature

Growth through the year stands in some relation to the temperature, and it appears to cease altogether when the daily mean air temperature is about 36° F. A comparison made between the extensions during the periods and the sum of ° F. over 36° (data are given in Table 3) gives a correlation ratio of + 0·816. This is high, the more especially as the temperature data are from Elveden Hall which stands 130 ft. O.D. and is 4 miles distant from the area under investigation, which, lying low, would be more likely to have frosts.

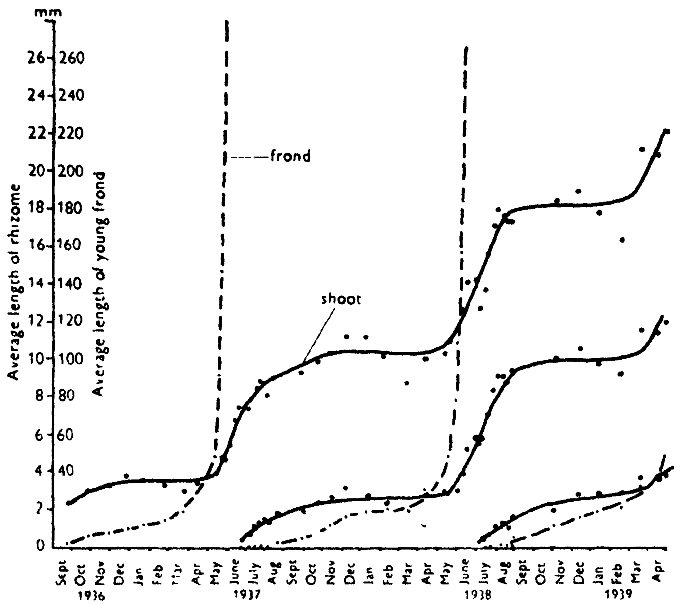


Fig. 10. The extension growth of the short shoot and the young fronds over the period August 1936 to May 1939. Based on data given on p. 420.

The growth of the short shoot and of the young frond

At intervals over a period extending from August 1936 to May 1939, 10 short shoots were dug up on each date in the uniform dense bracken of D to find out the time of the year when the rhizome and the young frond grow. Data concerning the frond should be considered more appropriately in the next section, but for convenience the data and graphs are given here. The rhizome was measured from the apex to the axils of the successively older frond insertions, and internodal lengths can be obtained by subtracting the figures in adjoining columns. Since the data were obtained from shoots which varied both in age and vigour there is much variation. A much larger number than 10 would have been required to obtain a proper average and an attempt to smooth out the curve was made by plotting the averages of each successive three sets of data (Fig. 10). The actual data are given in Table 4.

Table 4. *Length in cm. of the rhizome and fronds based on the average of* 10 *samples collected on each date over the period August* 1936 *to May* 1939. *The data for the rhizome are measurements from the apex to the axils of the fronds*

	Rhizome			Frond		
25. viii. 36	0·14	—	—	0·06	—	—
22. ix. 36	0·24	—	—	0·35	—	—
26. x. 36	0·30	—	—	0·49	—	—
24. xi. 36	0·35	—	—	1·09	—	—
22. xii. 36	0·34	—	—	1·02	—	—
21. i. 37	0·45	—	—	1·16	—	—
22. ii. 37	0·30	—	—	1·66	—	—
22. iii. 37	0·27	—	—	1·74	—	—
16. iv. 37	0·32	—	—	2·05	—	—
14 v. 37	0·47	—	—	6·66	—	—
20. v. 37	0·40	—	—	5·50	—	—
27. v. 37	0·58	—	—	15·37	—	—
3. vi. 37	0·44	—	—	23·06	—	—
12. vi. 37	0·63	—	—	45·01	—	—
19. vi. 37	0·98	0·06	—	71·40	0·01	—
26. vi. 37	0·62	0·03	—	73·30	0·02	—
5. vii. 37	0·64	0·06	—	83·80	0·03	—
14. vii. 37	0·97	0·13	—	113·1	0·06	—
21. vii. 37	0·93	0·18	—	119·6	0·10	—
29. vii. 37	0·74	0·11	—	118·9	0·05	—
4. viii. 37	0·94	0·16	—	117·6	0·08	—
17. viii. 37	0·74	0·14	—	113·9	0·14	—
28. ix. 37	1·03	0·24	—	—	0·50	—
22. x. 37	1·03	0·19	—	—	0·77	—
12. xi. 37	0·92	0·29	—	—	1·21	—
7. xii. 37	1·18	0·32	—	—	1·48	—
13. i. 38	1·30	0·35	—	—	2·40	—
13. ii. 38	0·87	0·18	—	—	1·67	—
11. iii. 38	0·89	0·19	—	—	2·36	—
13. iv. 38	0·86	0·25	—	—	1·74	—
12. v. 38	1·27	0·39	—	—	5·12	—
20. v. 38	0·99	0·26	—	—	3·27	—
2. vi. 38	1·08	0·31	—	—	6·47	—
9. vi. 38	1·19	0·34	—	—	13·91	—
16. vi. 38	1·53	0·53	—	—	26·86	—
30. vi. 38	1·53	0·71	—	—	37·05	—
7. vii. 38	1·24	0·55	0·03	—	44·06	0·03
15. vii. 38	1·08	0·46	0·06	—	50·85	0·04
21. vii. 38	1·84	0·75	0·05	—	47·49	0·11
28. vii. 38	1·77	0·93	0·11	—	70·48	0·19
4. viii. 38	1·55	0·85	0·17	—	76·16	0·12
11. viii. 38	2·08	0·97	0·06	—	—	0·08
20. viii. 38	1·71	0·93	0·19	—	—	0·12
27. viii. 38	1·43	0·73	0·04	—	—	0·04
4. xi. 38	2·09	1·20	0·29	—	—	1·70
14. xii. 38	2·06	1·10	0·28	—	—	1·74
13. i. 39	1·56	0·87	0·26	—	—	1·45
17. ii. 39	1·75	0·96	0·32	—	—	2·79
21. iii. 39	1·62	0·96	0·29	—	—	2·22
15. iv. 39	2·98	1·55	0·52	—	—	4·93
28. iv. 39	1·70	0·95	0·29	—	—	2·97
12. v. 39	1·99	1·12	0·36	—	—	6·77

The growth of the rhizome and of the young frond are presented graphically in Fig. 10. The differentiation of the apex into rhizome and visible frond takes place about the end of June (1937 was an exceptionally early year and free from damaging frosts) when the frond appears as a little squat lump. Thereafter the rhizome grows slowly but steadily well towards the end of the year, when growth almost ceases, to

be renewed again in May. Incidentally, inspection of the curves suggests that the internode behind the newly visible frond continues to grow slightly even after the appearance of the frond. Extension of the short shoot appears then to take place concurrently with that of the long shoot.

As to the young frond its growth rate is at first slow but is maintained through the winter: in April it grows must faster to emerge from the soil and grow at an average rate of 2·2 cm. per day between 3. vi. 37 and 14. vii. 37 and 1·15 cm. per day between 9. vi. 38 and 28. vii. 38. The difference between these two years is to be correlated with temperature: in 1937 there was practically no frost, but in 1938 frond growth was checked by frost damage.

In connection with the frond it should be noted that this account differs from previous accounts (Hofmeister, 1862; Klein, 1884) in showing that in this area at least the frond grows to maturity about 12 months after its first visible appearance (Fig. 7*c*).

The age of the bracken plant

It is commonly stated that the frond-bearing rhizome normally sends up one frond per annum and consequently adds one internode to its length. If this were so, the number of internodes would be a ready means of finding out the age of the short shoot and the long shoot bearing it. It is, however, an unreliable criterion, for a terminal bud may remain dormant for at least a year or may produce two fronds and two internodes in one year. The production of two internodes, however, is not common and not a source of grave error where large numbers are dealt with. On the other hand, dormancy is widespread, at least in E, and must introduce serious errors in estimates of age by this method, for it is unknown how long a bud may remain dormant or how often dormancy occurs in the lifetime of a shoot. Reference to Table 5 shows the difficulty. The data show not only the small number of fronds already alluded to but also the very high proportion of dormant buds even in the

Table 5. *The number of dead and live shoots and of live shoots with and without the current year's fronds in E*

	The terminal segment of the rhizome advancing into grassland E. Dug up August 1937	Lateral *X* (Fig. 5*a*). Dug up August 1937	Complete plant in the hinterland (Fig. 5*b*). Dug up July 1937
No. of dead shoots	0 (0)	37 (18·0)	20 (17)
No. of live shoots bearing current year's fronds	7 (18·5)	12 (6·0)	11 (9·5)
No. of live shoots *without* current year's fronds	27 (71·0)	105 (51·5)	77 (66·5)
Shoots accidentally cut	4 (10·5)	50 (24·5)	8 (7·0)
	38 (100)	204 (100)	116 (100)

rhizome spreading into new ground. (Since these data refer only to terminal buds and do not include dormant "frond-base" buds, the enormous reserve of buds becomes evident.) The number of shoots cut accidentally during excavation makes comparison difficult, but even if all these carried fronds the percentage of shoots

with fronds would still be much less than those without, and a proportional distribution of the cut shoots among those intact does not materially alter the percentage data given. It is clear therefore that an estimate of age cannot be obtained by a count of the internodes: nor do these data tell us how long a bud may remain dormant.

If we assume that an internode represents a year's growth, the number of internodes will give the *minimum* age of the short shoot and of the rhizome bearing it. Using this criterion we can at least say that the rate of growth in the hinterland of E may be extremely slow, for in Fig. 6*c* the eighth upright shoot at a distance of only 74 in. (1·88 m.) from the apex has 32 internodes. This represents an average rate of growth of the main rhizome of little over 2 in. (5 cm.) per annum. The seventh and sixth shoots from the apex had respectively 26 and 24 internodes. There is, however, no absolute regularity, for the third and second had only 3 and 5 respectively—and both these shoots were dormant—while the last, which from the fresh tomentum it carried appeared to have grown in one year, had 7 undeveloped lateral buds. The total length of the branch was 98 in. Its minimum age is therefore about 50 years: it cannot be much less and it may well be twice that. On the other hand, this extremely slow growth may not be fully representative, for, while no detailed records were made of other branches at the time, the field notes suggest a faster rate than 2 in. per annum. But no exact measurements have been made of rate of growth in this part of E.

On this question of age we may use the data already given to get approximate values for the plants invading grasslands D and E. In D the average annual extension in the 3 years 1936–7, 1937–8 and 1939–40 is 74·2 cm. No exact data on the length of the main axis of a plant stretching across the wave of bracken advance in D are available, but digging shows dead main rhizomes at 85 ft. (26 m.) from the front margin. On this basis the bracken plant would be about 35 years old at the dying end. In E on the basis of the average length of two main rhizomes (78 ft., 24 m.) and an annual extension of 33 cm. the age is about 72 years. These results are only approximations, but they give some idea of the time scale involved when we are dealing with bracken and of the need for carrying on experiments over an extended period in any attempt to present experimental proof of any hypothesis formulated to explain some of the phenomena described at the beginning of this paper.

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NOTE ON SPORE LIBERATION IN THE MUCORACEAE

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(With 1 figure in the text)

It is generally assumed that the spores of the Mucoraceae are in most cases air-borne, and that when the sporangium dehisces the spores, left exposed on the columella, are capable of being blown away by wind. Recently, however, Dobbs (1939) has observed that "when the sporangium bursts in air...the spores are not set free when the wall breaks, nor for some time afterwards. The sporangial wall is replaced by a water surface, and the sporangium becomes a 'sporangial drop' of liquid containing the spores and columella." He goes on to say, "The precise conditions under which spores are distributed into the air have not been determined, but there is no evidence that in any of the species examined the spores are so dispersed while the sporangiophore is still turgid." My own observations fully confirm those of Dobbs. It seems of interest, however, to decide whether spores are liberated from the dehisced sporangium directly into the air, or whether they only escape into the air when the whole mycelial mat, including the sporangiophores, dries up and dust particles with adhering spores are formed from it.

In investigating the ease with which spores become detached and escape into the air, the following simple method was used. The fungi, instead of being grown in ordinary Petri dishes, were cultured in deeper dishes of such a depth that there was always a space of at least an inch between the ends of the tallest sporangiophores and the lid of the dish. When a culture was at the required stage of development, it was turned upside down and the bottom of the dish tapped energetically with the finger. With this treatment spores which were free to escape might be expected to fall off into the air and collect on the lid of the dish. After the lapse of a few minutes, to allow time for liberated spores to settle, the Petri dish lid was removed, and its inner surface searched for spores under the microscope.

In the case of species of *Mucor* (*M. hiemalis* and *M. racemosus*) no spores were found on the lid after this treatment although cultures of different ages (1, 2, 3 and 5 weeks old) were used, nor was it found possible to blow spores from sporangia even after the sporangial drops had completely dried. Again in *Phycomyces nitens*, no spores could be jerked out of the sporangia at any stage. Indeed, in this fungus the sporangia appear to dehisce only where they come in contact with the wall of the culture dish. Then the spores, immersed in mucilage, become firmly cemented to the glass. Otherwise the sporangia, on finally drying, shrivel without ever bursting. In *Mucor* the sporangium wall breaks down in water. This happens naturally since

the apex of the sporangiophore usually secretes a drop of water which involves the sporangium, and a sporangial drop is formed as described and figured by Dobbs. In *Phycomyces* the wall is persistent, and although the sporangium usually bursts if it comes in contact with water, the wall does not break down.

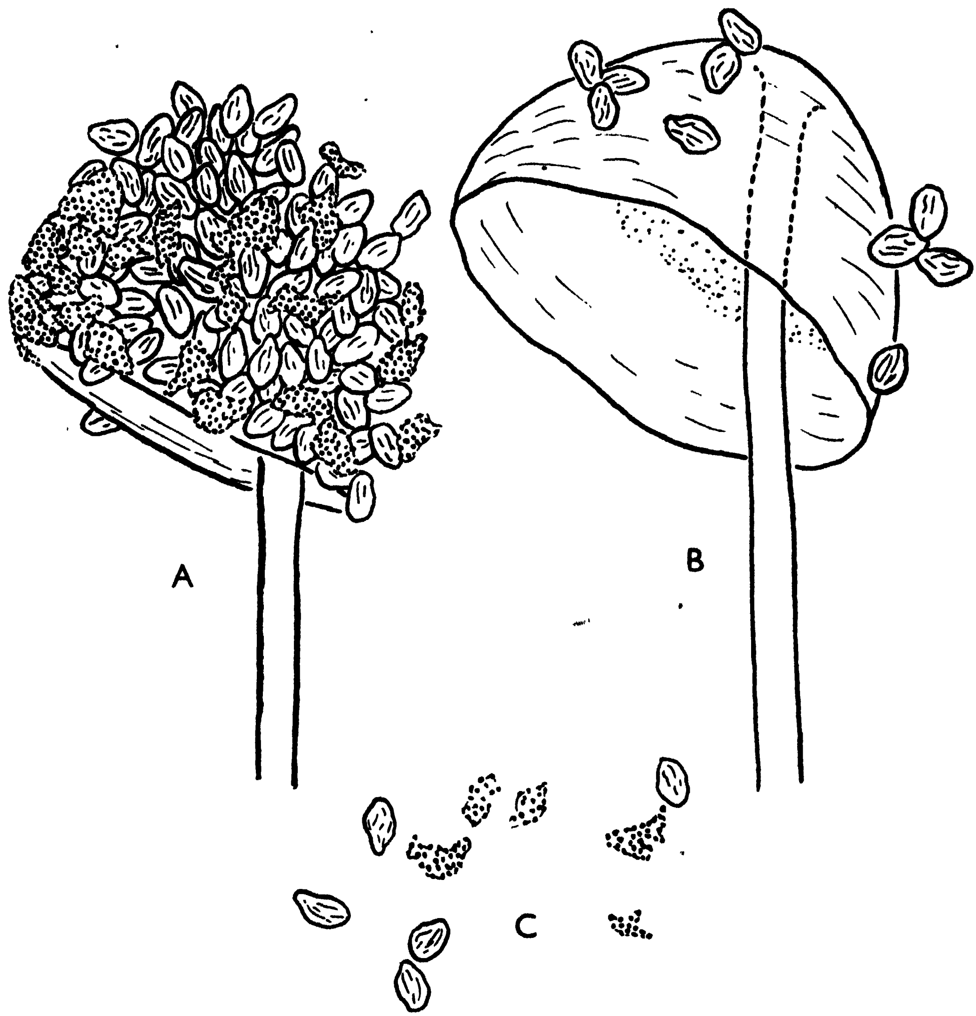

Fig. 1. *Rhizopus nigricans*. A. Dehisced sporangium projecting into the air. The collapsed columella bears a mass of spores and fragments of the sporangium wall. Many of the spores have already been blown away. B. A similar sporangium from which nearly all the spores have disappeared. C. From a spore deposit collected on the lid of an inverted culture. The spores, being dry, have a wrinkled appearance. × 500.

In *Rhizopus nigricans* tapping the inverted cultures (a week or more in age) led to the formation of a considerable spore deposit on the Petri dish lid. This deposit included fragments of the sporangium wall as well as the spores. In most of the Mucoraceae the sporangiophore relies for its rigidity mainly on turgor, but in *Rhizopus nigricans* the sporangiophore wall is rigid, and the relatively short sporangiophore

remains erect after the hydrostatic pressure within it fails. When this occurs the columella collapses, but in a definite manner. When turgid it forms a balloon-like swelling at the end of the sporangiophore, but when its turgidity is lost it collapses so that it looks like an inverted bowl resting, often slightly askew, on the end of the sporangiophore. The powdery mass of wall fragments and spores (Text-Fig. 1) exposed on this collapsed columella is fairly easily blown away.

In the genus *Thamnidium* the sporangiophore bears a terminal sporangium of the *Mucor* type, but lower down, from the axis of the sporangiophore, highly branched laterals grow out, each terminated by a small sporangium (sporangiole) containing four spores. On tapping inverted cultures of *Thamnidium elegans* no spores escaped from the terminal sporangia at any stage, but a deposit, although not a dense one, of sporangioles was obtained. It would seem in this case that the four-spored sporangiole acts as a single dispersive and reproductive unit which can fairly readily escape into the air and be dispersed by the wind.

In *Cunninghamella elegans*, one of the conidial forms of the Mucoraceae, tapping an inverted culture gave a thick deposit of spores on the lid of the dish, and it can also be shown very readily by direct microscopic examination that the spores are easily blown from the spherical heads.

In those species of the Mucoraceae which I have examined the *Mucor* type of sporangium can only be considered as an apparatus liberating air-borne spores in *Rhizopus nigricans*. The question arises, therefore, as to how the spores of *Mucor* are normally dispersed. There seems to be no definite evidence on this subject, but it may be that insects play a bigger part in dispersal than is generally supposed.

REFERENCE

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NOTE ON A SIMPLE POROMETER CUP FOR CLASS USE

By L. J. AUDUS
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(With 1 figure in the text)

DURING the course of classes in plant physiology to Intermediate and Final degree students it was thought desirable to design a simple form of porometer cup which could be firmly and rapidly attached to a leaf, and which would obviate the use of glue, vaseline, clamps and such apparatus which in the past has mutilated many a leaf. The modification of the familiar porometer cup shown in the illustration was therefore introduced and has been found very satisfactory.

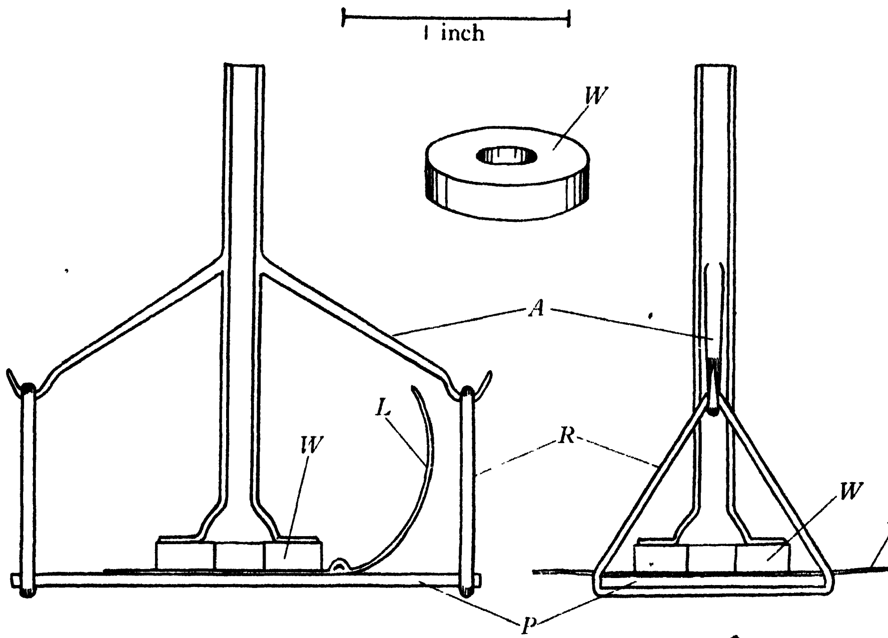


Fig. 1.

To the stem of the cup are fused two arms (A) provided with small hooks at their extremities. The junction between the leaf and the ground flange of the cup is made by a washer (W) of 30% gelatine about 2–4 mm. thick. The gelatin should contain a small quantity of thymol to keep it sterile. The washer can be conveniently cut by cork-borers of suitable size, from gelatin plated out in a Petri dish. The cup, washer and leaf (L) are kept firmly in contact by placing a narrow glass plate (P) under the leaf, and fastening it at the ends by the rubber bands (R) passing round the hooks on the lateral arms. Accurate construction of these arms and use of elastic bands of equal size ensure a symmetrical pressure of the cup and washer on the leaf surface and thus leaks are rare. The fixing of this cup on to the leaf takes only a few seconds.

LINE ILLUSTRATIONS

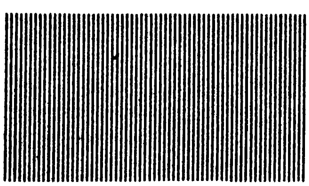
At the present time it is more than ever necessary to make use of the most effective and suitable methods of illustration. For the great majority of scientific purposes this is achieved by the use of line engravings on zinc. The special advantages of this method, which do not seem to be fully realized by many authors, include greater clarity of detail, more freedom of emphasis, and a better harmony when lettering is necessary than can ever be achieved by half-tones.

The impression that a line-drawing for reproduction is more difficult than photography is not borne out by editorial experience; a much lower percentage of bad drawings is received than bad photographs.

Where a wealth of detail makes drawing unduly laborious, a good photograph may be desirable, as in landscapes for vegetation studies and low-power sections of extensive tissues. The time taken in line-drawing may be much reduced by the use of mechanical tints which may also improve the appearance of the reproduction. A set of patterns of mechanical tints which are available for use in this *Journal* is given below. They are particularly useful in the preparation of maps, vegetation and pollen diagrams and for graphs with shaded areas; but they can be used for any form of shading whatever.

In ordering them, the area to be shaded should be left plain white; and its boundary lightly indicated with a blue pencil line, if the border-line is not to appear in the reproduction. Within the area marked, the tint desired should be indicated, also in blue pencil, by its number in the accompanying patterns. Where the tint consists of parallel lines it should be stated whether they are required vertical, diagonal or horizontal. The coarser patterns usually reproduce better than the very fine ones.

Patterns



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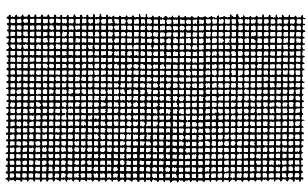

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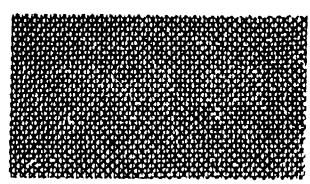

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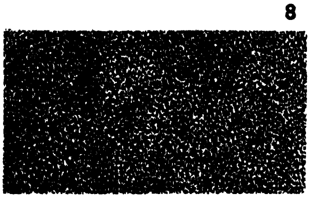

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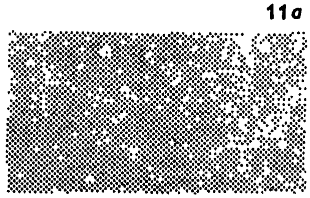

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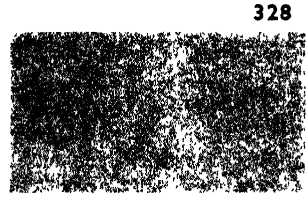

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Copies of this list may be had free on application to the Editors.

REVIEWS

Die Geschichte der Moore des Sihltales bei Einsiedeln. By WERNER LÜDI. 9 × 6 in. Pp. 95, 22 text-figs. and 9 plates. Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich, Heft 13. Hans Huber, Bern, 1939. Price 7.50 frs.

Before its conversion into a reservoir in the spring of 1937, the Sihltal on the north flank of the Glarus Alps was famous for the flora of its peat bogs. Dr Lüdi has now put together the results of his substantial investigations of the stratigraphy of the bogs and the history of the valley deposits. The valley surface, which is now about 900 m. above sea level, at the end of the glacial period carried the great Sihl Lake, which was fed by the streams of the Sihl and the Minster entering at its southern end.

Pollen diagrams from the bogs along the narrow valley show general agreement with one another, and from them is deduced the general forest sequence: *Pinus*, *Corylus-Pinus*, *Corylus-Quercetum Mixtum*, *Abies*, *Abies-Picea*, *Abies-Picea-Fagus* (*Abies-Picea*), *Picea-Pinus* (to surface). There are two clear periods after the pine period; the earlier with dominant *Quercetum Mixtum* (chiefly *Ulmus* and *Tilia*) and *Corylus*, and the later with *Abies*, *Picea*, and *Fagus*. *Alnus*, mostly the mountain-side shrub *Alnus incana*, is an important component from the early *Corylus* time. *Betula* pollen is unimportant, and where frequent is referred to *B. nana*. Sites at the northern end of the valley are open to the lower mountain slopes, and received great amounts of pollen from the beech and mixed-oak forests growing there, but the southern end of the valley, further into the high mountains, has sites which reflect the greater influence of subalpine woods, and their diagrams show more spruce and fir pollen.

The evidence of the pollen diagrams shows that the peat deposits at the southern end first formed in the pine period. Samples taken successively northwards along the valley proved the organic deposits to have had a later and later origin. In this way the author has been able to reconstruct the course of progressive northwards filling up of the former lake by gravel, sand, and silt brought down by the rivers. The great acceleration of this process in the *Abies* period is thought to point to an increased precipitation at this time.

The bogs have not yielded the means of direct correlation with prehistoric cultures, and so the forest periods have been dated by comparison with the sequence in other sites nearby. The broad climatic inferences are as follows: The *Abies* period is indicative of increased wetness, the extension of *Picea* is attributed to cooling, and the chief extension of *Fagus* in this moist mountain region is held to indicate the modified effect of the dryness of the Bronze Age. A magnificent aerial photograph of the valley and other excellent views complete the book, which constitutes another step towards completion of our picture of post-glacial forest history in relation to climatic and geological change. H. GODWIN

Starch and its Derivatives. By J. A. RADLEY. $8\frac{1}{2} \times 5\frac{1}{2}$ in. Pp. x+346, with 61 microphotographs and 28 figures in the text. London: Chapman and Hall. 1940. Price 22*s*.

This monograph is published in a series devoted to applied chemistry and is mainly concerned with technical processes and applications. There are, however, several sections of direct interest to botanists. One of the most obvious of these is the collection of microphotographs of starches from numerous sources under direct illumination; with the Spierer lens; and between crossed Nichols. It is pointed out that, while these plates are useful for preliminary identifications, reasonable certainty can only be achieved by direct comparison with authentic samples. Further methods of examination and analysis are also dealt with in some detail.

An up-to-date account is given of the organic and physical chemistry of starch and its derivatives and also something of the starch-degrading enzymes. The fundamental unit of the starch molecule is accepted as α-glucopyranose. These units are joined by a 1–4 carbon atom linkage, as in maltose, imposing a spiral twist on the thread-like molecule. The chain length is regarded with more doubt, and solutions of the problem are given varying from 24 to 1770 glucose units. The position of the phosphate radicals is also considered to be undecided.

The more technical sections of the work deal with the manufacturing processes of extrac-

tion and utilization. The main commercial sources of starch are rather surprisingly restricted to a mere half-dozen—potato, sweet-potato and tapioca; and the cereals, maize, wheat and rice. Conversely, the uses of starch are remarkable for their variety and number. Starch is the main commercial source of maltose, glucose and dextrins, and an account is here given of the accidental discovery of British gum following a fire in a Dublin textile mill. Alcohol, acetone and vinegar are also commercial starch products. Pastes and vegetable glues (after treatment with alkali) are well-known uses of starch itself, as well as a whole range of powders used in cooking, confectionery and cosmetics. It is a much-debated point whether the swelling of starch grains used in face powders is likely to enlarge the pores and coarsen the skin. Somewhat less familiar uses of starch occur in the manufacture of explosives, dry batteries and luminous paint. All these and many others are described in this volume. W. O. JAMES

Elementary Microtechnique. Second Edition, by H. A. PEACOCK. $7\frac{1}{2} \times 4\frac{1}{2}$ in. Pp. viii + 330, with 21 figures in the text. London: Arnold. 1940. Price 9*s*.

This is a new and considerably enlarged edition of a useful and well-established handbook whose merits have already been described in this *Journal*, 34, 262. New additions include a graded selection of type methods intended for the instruction of beginners and a simple method of dark-ground illumination. Considerable use has been made of suggestions and information offered by users of the first edition. A sound change in the new issue has been the systematic use of the nomenclature adopted by the United States Commission on Standardization of Biological Stains. As a measure of reinsurance, the Colour Index Numbers of the British Society of Colourists are given synonymously. W. O. JAMES

The Grasslands of the Falkland Islands. By WILLIAM DAVIES. $9\frac{1}{2} \times 6$ in. Pp. 86, with 18 photographs and 2 maps. Crown Agents for the Colonies, 4 Millbank, London, S.W. 1. 1939. Price 5*s*.

The group of islands known as the Falklands lies about 300 or 400 miles east of the southern tip of South America. They have a total area of about 3,000,000 acres, and a good deal of this is mountainous. Although they have the latitude of mid-Wales, the winter temperatures are somewhat lower and the summers considerably cooler. The climate is very variable from day to day, and the rainfall (of the order of 25 in. per annum) well distributed through the year. Snow comes often on the hills, but lies only a short time. Strong winds are very frequent.

The islands carry a sheep population of about 6,000,000, and the 2500 people live mainly by sheep farming. The country has much in common with the hill country of Wales, and ecologists will recognize the good sense which sent Mr Davies from the Welsh Plant Breeding Station at Aberystwyth to the Falklands to make an agricultural survey of the islands and of the problems of grassland management there. The need for scientific advice was indicated by the slow but progressive decline in sheep population since the turn of the century and the low average carrying capacity of the land, which now only supports about one sheep per 5 acres.

The author regards the natural climax vegetation as grassland, but mentions two native bushes which may once have been more important.

A feature of much interest is the belt of "tussac grass" (*Poa flabellata*) which extends, a few hundred yards wide, round the coasts of all the islands, where its presence may be determined by salt spray borne inshore by the wind, or by the high nitrogen of a dense seal and sea-bird occupation. The suggestion is made that this plant might, under suitable treatment, become a valuable source of winter feed for sheep, although it rapidly disappears under uncontrolled sheep grazing. *Empetrum rubrum* also covers large areas which tend to be near the coasts. Stockyards, sheep pens and penguin rookeries have pasture almost entirely dominated by *Poa annua*, an introduced species. Mr Davies points out the effects of selective grazing in causing overstocking on the dry ridges bearing *Deschampsia flexuosa* and *Festuca ovina* (native variety), and of the fertile, narrow valley floors which are dominated by *Juncus scheuchzerioides*. The "white grass", *Cortaderia pilosa*, "enters practically every association, and is the dominant element in most plant communities", but the green, closely grazed

pastures near the settlements are composed of introduced bent (*Agrostis* sp.) and meadow grass (*Poa pratensis*). The author joyfully records the high productivity of wild white clover leys, and, as one might expect from Sir R. G. Stapledon's staff under these conditions, recommends their much extended use. "The greatest single factor in the pursuit of land improvement in the Falklands is the spread of wild white clover." At present the clovers spread only vegetatively, for the absence of bees precludes the setting of seed. It is recommended that suitable bees should be introduced to meet this difficulty. Bacterial inoculation will also be necessary, for appropriate nodule formers are practically absent from the natural soils.

Where the surface is peat-covered the ground is spoken of as "soft camp", and typically this bears an association dominated by *Cortaderia pilosa* and *Oreobolus obtusangulus*. On very deep peat *Rostkovia magellanica* and *Astelia pumila* also enter. For such areas reclamation by drainage, burning, and resowing is advocated. The author describes the results of seeding and manuring trials organized from Aberystwyth in 1935–6. Cocksfoot (*Dactylis glomerata*) and Yorkshire fog (*Holcus lanatus*) were the introduced grasses showing most promise, and *Plantago lanceolata*, *Lotus major* and *Achillea millefolium* also did well.

The manurial treatments gave rather perplexing results. In spite of low lime content there was no evidence of beneficial influence of liming, nor was phosphate manuring very effective. Everywhere a primary factor in amelioration is held to be the destruction of the tough peaty mat of undecomposed plant remains which covers the soil. We hope it is recognized how great the water-conserving powers of this mat will be, and trust its removal may not lead to the drastic soil erosion which has followed removal of the natural vegetation mat in so many other places. Organic manuring with offal and carcases had extremely great effects, comparable with those caused by animal droppings on spring and winter pastures in England. High nitrogen supply and particularly the growth of clover are shown greatly to improve biological soil activity, and promote the mat destruction.

Chemical analyses of native and introduced plants grown in the islands were made in Aberystwyth, and the tabulated results are seen to bear closely on the problems of a revised pasture management. The high protein, phosphorus, potassium and calcium content of two kelp samples is rather striking in view of the suggestion that in winter the Falkland sheep eat the seaweed cast up on the beaches.

Although the volume is written in a popular style with a view to its use by the island sheep farmers, enough will have been said to indicate the interest that it will hold for botanists. The book contains a simplified vegetation map of the islands and eighteen photographs illustrating their vegetation.

H. GODWIN

Plant and Animal Communities. Edited by THEODOR JUST. $9\frac{1}{4} \times 6\frac{1}{4}$ in. Pp. 255. Reprinted from the *American Midland Naturalist*. The University, Notre Dame, Ind., U.S.A. 1939. Price $2.50.

At the end of August 1938 a number of prominent ecologists, mostly American, held a symposium at Cold Spring Harbor, New York, to discuss the "status and delimitation of the fundamental entity of community study". It was "conceived and directed" by Dr S. A. Cain. This book is a record of what was said at the conference, and we cannot better indicate its scope than by giving the names of the contributors and the titles of their papers:

H. S. Conard: Plant Associations on Land.
G. E. MacGinitie: Littoral Marine Communities.
F. E. Eggleton: Fresh-Water Communities.
J. R. Carpenter: The Biome.
H. A. Gleason: The Individualistic Concept of the Plant Association.
T. Lippmaa: The Unistratal Concept of Plant Communities (The Unions).
S. A. Cain: The Climax and its Complexities.
A. E. Emerson: Social Coordination and the Superorganism.
N. Tinbergen: On the Analysis of Social Organization among Vertebrates, with special reference to Birds.
T. Park: Analytical Population Studies in relation to General Ecology.

These papers have long and useful bibliographies, and the discussion which followed the reading of each contribution has been shortly reported. The condensed discussions are

interesting because they reveal, as so often do verbal comments at the conclusion of scientific addresses, a considerable wariness in agreement, and not seldom an entire failure to accept the speaker's arguments. This divergence strengthens the feeling of cleavage between the different contributors which is expressed in their papers, and which warrants the editor's remark: "No aim at exhaustive treatment is claimed but rather a deliberate effort to present a cross section of current concepts with suggestions for future work." He adds that the book "tries to bridge the wide gaps existing particularly between cis- and trans-atlantic ecology, between the botanical and zoological aspects...", and even if such bridges have not been built, at least we may say the contiguous territories have been partly surveyed.

No ecologist can read the book without interest and profit, but for any agreement as to the status of fundamental vegetational units he will look in vain. Let the student read this book to see the emphasis and interpretation other ecologists have found of value in their own work, let him consider if their ideas illuminate his own problems, but do not let his aim be limited to squeezing the results of his own vegetation studies into any one of these developed schemes, for his own sample of organisms will be organized in its own pattern and have its own interpretation. The more one sees of these discussions on vegetational units the more it appears that so long as we engage in resolving the patterns of organization in communities, and discovering the mechanisms by which structure is established, maintained, or altered, we shall be dealing with matters of general applicability capable of direct linkage to fields of experimentation and the wide existing knowledge of plant and animal biology. On the other hand, our time is likely to be wasted, if, before these results are clear, we seek by discussion alone to determine the elusive (and perhaps illusory) "fundamental units of community study".

We are slowly recognizing that the concepts of each ecologist are constructed in relation to the region in which he has himself worked, and that there must be regional communities of ecologists as of other organisms. All the word-making and definition of concepts without general agreement which this book reveals must surely mean that in the hierarchy of plant and animal communities there is an almost endless variation of kind and degree of integration, and of interval between one community and its neighbour. The units and factors which we rightly stress in one place are of less importance in another.

The authors in this volume are naturally of two types, those who describe their own work and on the basis of it press for a recognition of a new order of integration (for instance, Lippmaa and his "Unions"), and those who simply review the scope of current ideas in one section of the field of syn-ecology. In the latter class there is much tiresome reading and repetition of phrases that ought to be criticized out of countenance. Now is hardly the time for such criticism, but whatever *can* be meant by Clement's phrase: "Competition occurs wherever two or more plants make demands in excess of the supply" (p. 149)? And if a sere is a change in time, how can a clisere be a "series of climax formations or zones which follow each other in a particular climatic region subsequent to a distinct change of climate"? Surely zones, which are units spatially separated, can never be seres, although they may be indices, more or less trustworthy, to seres.

In the welter of notions flung abruptly against one another in this compendium it is a great pleasure to read the restatement by Gleason of his "Individualistic Concept of the Plant Association". As one forced to see, in repeated studies of bog stratigraphy, how rapidly climatic change can affect the direction of vegetational development, and how greatly species and communities alter their range within a few thousand years, the reviewer welcomes Gleason's stress of the ever-changing character of vegetation. In his paper alone we are given no bibliography: it isn't needed, and the author no doubt felt, as others will, that he still stands on his own feet as he did twenty-four years ago when he first put forward these views, and that his criticisms are still mostly unanswered. This is not, of course, to say that Gleason's ideas give us the whole story of the interpretation of plant communities, far from it, but they do represent healthy critical standards in our studies of these entities.

There is on pp. 147–8 an attempt to limit the use of "community" to certain categories of vegetation with certain properties. I for one shall go on using the term in a perfectly general way for any sized group of living organisms so long as they have any kind of integration. Are we to have left *no* general term for all vegetational units, and are we to have left *no* common terms in which the common and commonsense usage of English may be preserved?

H. GODWIN

The New Systematics. Edited by JULIAN HUXLEY. $8\frac{1}{2} \times 5\frac{1}{2}$ in. Pp. viii + 583, with text-figures and tables. Oxford: Clarendon Press. 1940. Price 21*s.*

This volume, a collection of papers by twenty-two distinguished biologists, was produced under the auspices of the Association for the Study of Systematics in Relation to General Biology. The Association, and especially the Editor, are much to be congratulated. The contributions vary a good deal in length and cover a considerable range of topics, but the standard is extremely high throughout, with the result that biologists have a very full and authoritative account of the facts, ideas and arguments which underlie and constitute the new approach to taxonomy. The first paper, by the Editor, is an admirably balanced summary of the rest of the book, welding the most diverse material into a coherent whole. Any selection of papers for individual mention must be a very personal matter, but three by eminent foreign biologists seem of special interest: those by N. Timoféef-Ressovsky, by H. J. Muller and by Sewall-Wright. Of these, the first, dealing largely with problems of micro-evolution and geographical variation, concludes that we are sufficiently supplied with general fundamental principles and mechanisms for their explanation, but appeals for the intensive field-work which alone can give numerical values for the general formulae. This paper ends with the statement that "Anti-selectionist and anti-genetical evolutionary speculations have to-day no scientific value, even in connection with the problems of macro-evolution". Muller's account of the bearings of the *Drosophila* studies on taxonomic problems arrives at the interesting conclusion that in *Drosophila* racial and even specific differentiation depends almost entirely upon numerous gene mutations individually of small effect, structural differences in the chromosomes being of secondary importance. Here again stress is laid on the adequacy of the present framework of Mendelian and Selection theory to deal with taxonomic problems. Sewall-Wright's contribution summarises some highly relevant consequences of mathematical work on the mechanism of species differentiation, and illustrates the broadening of outlook and the increased precision of thought which a mathematical treatment has made possible.

Perhaps the most striking features of the volume as a whole are first the wide range of individual interests represented by the contributors; second the faith of most (though not quite all) contributors in the adequacy of the present theoretical structure based on Mendelian inheritance, mutations and selection; third, the increasing importance of the ecologist's contribution to taxonomy; and fourth, the Editor's suggestion that the term "variety" be dropped completely from the systematist's vocabulary, as having no definite meaning.

A. R. CLAPHAM

The Course of Evolution by Differentiation of Divergent Mutation rather than by Selection. By J. C. WILLIS. $9 \times 5\frac{3}{4}$ in. Pp. viii + 207, with 10 text-figures. Cambridge University Press. 1940. Price 12*s.* 6*d.*

In this book the author of *Age and Area* reviews a great body of facts which are, in his opinion, in conflict with the theory of evolution by the natural selection of minute adaptative variations. The facts must certainly be faced by evolutionary theorists and it is good to have them presented, but a misleading impression is given both of the magnitude of the difficulties and of the extent to which there has been failure in meeting them. The author quotes so little from the vast recent literature of cytogenetics and of mathematical selection-theory that he must be suspected of being unfamiliar with it. This unfamiliarity would explain his misconceptions as to the relation of the "Hollow Curve" to modern views on the origin of species. Accepting that the form of the curve demonstrates that when one genus gives rise to two, both usually survive, it does not follow that "natural selection can have had little or no influence in the matter". Unfamiliarity with recent literature would explain, too, the continual flogging of dead or moribund horses. Thus modern conceptions of the nature of inherited variation can deal with the apparent difficulty that "unless a great many individuals varied in the same direction over the whole of a considerable area, the improvement would be promptly lost by crossing". The existence in definable circumstances of a large amount of non-adaptive differentiation of local populations is, again, not only consistent with but a necessary consequence of modern selection-theory.

A. R. CLAPHAM

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